Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **28** (2): 173-82 (2009)

Original Article

Received: November 28, 2008
Accepted: December 9, 2008

Determination of Sulphate for Measuring Magnesium Sulphate in Pharmaceuticals by Flow Analysis-Fourier Transforms Infrared Spectroscopy

Fernando OVALLES [1]*, Máximo GALLIGNANI [2], Rebeca RONDÓN [2], María R BRUNETTO [2] & Rafael LUNA [1]

[1] *Analysis & Quality Control Department, Faculty of Pharmacy, University of Los Andes, Mérida 5101-A, Venezuela*

[2] *Chemical Department, Faculty of Science, University of Los Andes, Mérida 5101-A, Venezuela*

***SUMMARY*. The viability of employing flow analysis coupled to Fourier transform infrared spectroscopy (FA-FTIR) as a useful tool for quantitative measuring of magnesium sulphate in pharmaceuticals was explored, developed and validated. The method was based on mid-IR transmittance measurements of the peak-area belonging to the sulphate band around 1110 cm^{-1} and the use of an external calibration curve. Dynamic range was established over a concentration range from 1 to 50 $mg.ml^{-1}$, with a limit of detection (3σ) of 0.26 $mg.ml^{-1}$. The analytical frequency was 12 h^{-1} with a precision close to the unit (% RSD). The analytical results obtained in commercial formulations by applying the proposed FA-FTIR method were in strong agreement with labelled values and those obtained by a reference titration method at 95% confidence level. Among various advantages offered by the proposed method over conventional ones, simple strategy and clean analytical chemistry must been highlighted.**

INTRODUCTION

Sulphate-mineral salts are known to have a wide range of therapeutic and cosmetic properties. For instance, magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) is available over-the-counter generically and under several brand names, such as Epsom salt which may be used orally or as a rectal enema; the sulphate anion - at elevated concentrations- has a cathartic effect on humans [1]. It is also prepared as a sterile solution of $MgSO_4.7H_2O$ in water for injection, administered by the intravenous or intramuscular routes as an electrolyte replenisher [2]. It is the anticonvulsant of choice for both prevention and treatment of eclampsia. It is one of the medicines that should never be lacking in the public hospital emergency [3]. Current state of magnesium sulfate research and therapy has been reported elsewhere [4].

In general, all products used as therapeutic agents require accurate analysis in order to guarantee quality, efficacy and safety. In this sense, finished products containing magnesium sulphate have an official method to confirm that they contain the required amount of the active ingredient. The official assay to analyze magnesium sulphate in parenteral formulations is based on a complexometric titration with sodium edetate [5].

Obviously, a great variety of methods are available for the determination of both magnesium and sulphate. Magnesium ion has been determined by indirect molecular absorption spectrophotometry [6,7], ion chromatography [8,9], capillary electrophoresis [9], potentiometry [10], and atomic absorption spectroscopy [11], inductively coupled plasma mass spectrometry [12]. Similarly, determination of sulphate in diverse matrices has been carried out by using classical gravimetry [13], turbidimetry [14,15], ion chromatography [15,16], flow-based automatic analyzers [17-19], inductively coupled plasma atomic emission spectrometry [15], capillary electrophoresis [20-22], and so on. In most cases, each named method could be

KEY WORDS: Epsom salt, Flow analysis, Fourier transforms infrared, Magnesium sulphate, Pharmaceutical analysis.

* Author to whom correspondence should be addressed. *E-mail:* ovallesd@ula.ve

regarded as useful for analyzing magnesium sulphate in pharmaceutical products. However, most of them are involved with the consumption of reagents -some greater than others-, and some of them involve separation techniques. It is now widely accepted that the use of chemical reagents should be minimized for protecting human health and the environment [23].

In this fashion, our intention was to propose the use of flow analysis combined with transmittance FTIR measurements for the determination of magnesium sulphate -with a very simple matrix such as parenteral injections and Epsom salts-, keeping in mind the principles of the green analytical chemistry.

Infrared absorption spectroscopy has been a standard method of analytical pharmacy for a long time. It can provide analytically useful information on a large variety of compounds, ranging from small inorganic ions to large organic molecules. In the two last decades, FTIR spectrometric methods have emerged as an analytical tool for quantitative analysis of pharmaceutical products [24-26]. FTIR could play a key role for future quantitative pharmaceutical analysis owing to the advantages on working with this vibrational technique. The above referred advantages have been mentioned elsewhere [24-27].

Measuring aqueous solutions in the mid-IR (MIR) region is not straightforward since water absorbs intensely in this segment [27]. However, today are available commercial cells with micrometer optical-pathlength and water-resistant window materials which offer the possibility of obtaining transparency regions in the MIR, mainly in the fingerprint region (1500–950 cm^{-1}) [28]. An interesting review on developments of FTIR spectroscopy as detection principle in aqueous medium was published nearly a decade ago [27].

It is known that sulphate ion presents vibrational peaks in the finger print region [29,30]. In this sense, attenuated total reflection-FTIR has been used to study magnesium sulphate on both aerosols and diluted solutions [31], and diffuse reflectance-FTIR has been proposed to measure sulphate in environmental samples [29]. FTIR spectroscopy has also been used to determine sodium sulphate and sulphate composition of heteropolysaccharides base on a standard curve obtained from a linear plot of sulphate concentration versus the weight of the IR band area of S=O stretching [32].

The present article has been focussed towards developing an alternative methodology for the quantitative analysis of magnesium sulphate in pharmaceuticals by using the sulphate vibrational band obtained by means of transmittance FTIR spectroscopy. Additionally, a FA system was coupled to the FTIR equipment in order to avoid the high manipulation of the transmittance cell. The present work offers an attractive and competitive alternative when compared to any other method because reagents and sample pre-treatment (except dilution) are not required.

MATERIAL & METHODS

Apparatus

A Spectrum 2000 FTIR spectrophotometer (Perkin Elmer, Norwalk, CT, USA) equipped with a temperature stabilized deuterated triglycine sulphate detector, a KBr-Ge coated beam splitter and a globar IR source, was employed for FTIR spectra acquisition, using a sealed cell (Wilmad Labglass, Buena, NJ, USA) for liquid transmission having ZnSe windows (38 x 19 cm size and a 2 mm thick) and an optical pathlength of 0.05 mm. FTIR spectra and data treatment were recorded and processed using a Spectrum 2000 software (Perkin Elmer, Norwalk, CT, USA) for Windows. The FTIR equipment was coupled to a mono-channel flow system through a sealed cell, adopted for flow analysis. The employed manifold (Fig. 1) facilitated automatic filling and cleaning of the flow cell for the continuous-flow measurements. It was constructed with an Ismatec IPC peristaltic pump (Glattbrugg, Switzerland) equipped with Tygon and PTFE tubing. A Rheodyne manual selecting valve (Alltech, Waukegan, USA), was used for carrying either the sample/standard or the blank solution into the flow cell.

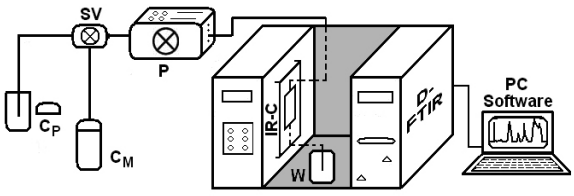

Figure 1. Schematic diagram of the FA-FTIR system. $\mathbf{C_P}$, blank channel; $\mathbf{C_M}$, sample/standard channel; **SV**, manual selecting valve; **P**, peristaltic pump; **IR-C**, transmission infrared-cell; **D**, detector; **W**, waste.

Samples and reagents

All chemicals used were of analytical reagent grade. Ultrapure deionised water (resistivity of 18 MΩ cm^{-1}) obtained from a Milli-Q-TOC sys-

tem (Millipore, USA) was used for the preparation of solutions. Magnesium sulphate heptahydrate of analytical grade (99.8% w/w, by titrimetric with disodium EDTA) was acquired from J.T. Baker (Xalostoc, Mexico).

Pharmaceuticals analyzed in this study were obtained from the Venezuelan market: (S1) Magnesium sulphate injection 50% w/v from Behrens Laboratory; (S2) Magnesium sulphate injection 12% w/v from Behrens Laboratory; (S3) Magnesium sulphate injection 12.3% w/v from Ronava Products for Biotecnoquímica Natural Medicines; (S4) *Epsom* salt from the Drotoca supplier; (S5) *Epsom* salt from the Misfarven supplier. *Epsom* salts are pure colourless crystals of magnesium sulphate heptahydrate. Magnesium sulphate injection is a sterile solution of magnesium sulphate heptahydrate in water for injection.

Reference procedure

The official assay method for magnesium sulphate based on a complexometric titration was followed according to the United States Pharmacopoeia Nº 29 [5]. This procedure requires several chemical reagents, such as sodium hydroxide, buffer solution of ammonium-ammonium chloride, eriochrome black T, disodium edetate (EDTA), apart from the EDTA standardization.

Standard/Sample preparation

In general, taking into consideration that the aim of this work was to propose a methodology for the analysis of magnesium sulphate in pharmaceutical formulations the concentration units were expressed as mg $MgSO_4.7H_2O$ mL^{-1}. In the proposed method, special sample preparation procedures were not required.

A stock standard solution of magnesium sulphate, with a concentration of 500 $mg.ml^{-1}$, was prepared by appropriated dissolution of the salt in water. Working standard solutions were freshly prepared each day and diluted as appropriate (5, 10, 20, 30, 40, and 50 $mg.mL^{-1}$, respectively) directly from the stock standard solution. Sample working solutions containing 25.0, 24.6 and 24.0 $mg.mL^{-1}$, respectively, were prepared by an appropriate dilution of the parenteral formulation. Solid samples commercialized as *Epsom* salts, from two different suppliers, were prepared at 500 $mg.ml^{-1}$ by appropriate dissolution in water. Working sample solutions were prepared at 25 $mg.ml^{-1}$ by appropriate dilution with water prior their FA-FTIR analyses.

The technique of standard addition consisted of adding 0.0, 1.0, 2.0, 3.0 and 4.0 mL of the stop standard solution (500 $mg.mL^{-1}$) to each parenteral formulation S1 - S3 (1.0, 4.0 and 4,0 mL, respectively), and then made up to 50.0 mL with water. On the whole, after appropriate dilution, samples were directly analyzed. All solutions were kept in a refrigerator at 4 °C until to be analyzed.

Study related to pH adjustment of magnesium sulphate solutions

A standard solution containing 50 $mg.ml^{-1}$ of magnesium sulphate heptahydrate showed a pH value of 5.9. Therefore, addition of sodium hydroxide and hydrochloric acid was required for pH adjustment between 5 and 8. After that, the standard solutions were finally diluted to obtain 25 $mg.mL^{-1}$.

FTIR procedure

Prior to sample/standard analysis, the flow system was conditioned by passing distilled water at a flow rate of 0.3 mL min^{-1} by using a peristaltic pump (Fig. 1). After 3.6 min, a background spectrum was established between 1600 and 800 cm^{-1} covering the selected analytical absorption-band of the sulphate anion. All spectra were recorded in the continuous-flow mode by co-adding 20 scans at 4 cm^{-1} nominal resolution. The time required for obtaining each spectrum under the stated conditions was approximately 1.15 min. After the background was established, the standards were propelled throughout the FTIR measurement flow-cell by switching the manual valve and using the peristaltic pump (Fig. 1). Once the calibration curve was plotted, each sample could be analyzed every 5 min. This period included filling of the flow cell, spectrum recording and cleaning of the flow cell. All of them carried out in a continuous-flow mode.

For quantification, a calibration curve was obtained from a linear plot of magnesium sulphate heptahydrate concentration versus the area of the IR absorption of sulphate at 1110 cm^{-1}. Baseline correction was established between 1185 and 1011 cm^{-1}. Data found for samples were interpolated in external calibration line established from the measurement of six standard solutions covering a concentration range from 5 to 50 $mg.ml^{-1}$ of $MgSO_4.7H_2O$.

RESULTS AND DISCUSSION

Performance characteristics such as linearity, accuracy, precision, dynamic range, detection

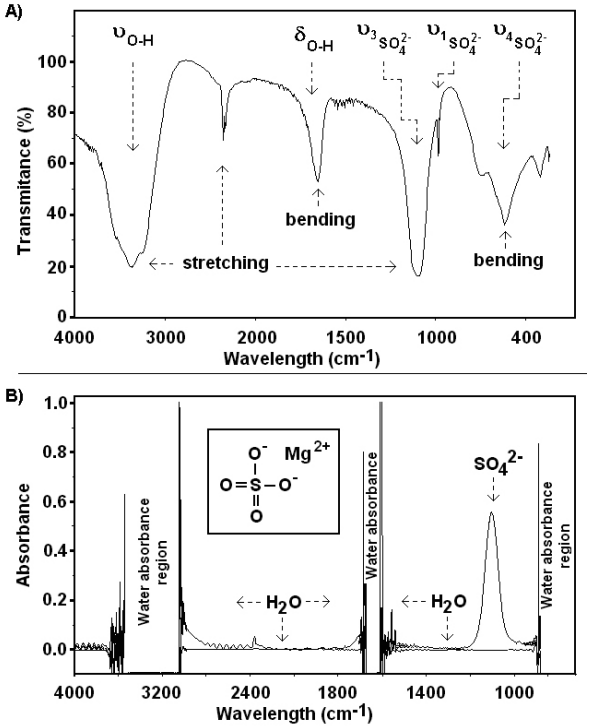

Figure 2. **A**) FTIR spectrum of $MgSO_4.7H_2O$ using KBr as background showing the absorption peaks found for sulphate under its magnesium salt. **B**) FTIR spectrum of $MgSO_4.7H_2O$ using water as background showing a single band in the fingerprint region. Spectra were recorded at a nominal resolution of 2 cm^{-1} with 25 co-added scans. Concentration of the sulphate standard corresponds to 25 mg $MgSO_4.7H_2O$ mL^{-1}. Peak assignment obtained from the specialized literature [30,33,34].

limit, quantification limit, specificity, recovery and robustness were studied. A mono-parametric study of the experimental and instrumental variables was performed and the selection of these was basically in terms of sensitivity, spectral acquisition time and precision.

FTIR peak identification of sulphate

The FTIR spectrum of magnesium sulphate heptahydrate (in solid phase) is shown in Figure 2A. The spectrum was obtained at a level of 10% (w/w) $MgSO_4.7H_2O$ spiked over KBr. The FTIR spectrum was interpreted following the characteristic IR absorption bands known for sulphate functional groups [30,33,34]. It shows the broad O-H stretching band in the 3000 to 3700 cm^{-1} range. The absorptions within 2265-2080 cm^{-1} correspond to O–H stretching vibrations of cluster of water molecules of crystallization. The bending modes of different water molecules of crystallization are observed from 1700 to 1555 cm^{-1} indicating that the solid is a hydrate. The strong and broad band with a maximum at 1110 cm^{-1} and the small shoulder at 998 cm^{-1} could be assigned to sulphate absorptions: ν_3 (asymmetric stretching vibrations) and ν_1 (symmetric stretching vibrations), respectively. The bending mode of SO_4^{2-} occurs at 618 cm^{-1}. The strong and broad absorption band at 613 cm^{-1} probably resulted from the combined absorptions of sulphate (ν_4) and other vibrations of the molecule. By concluding, the characteristic IR absorption bands observed (in solid phase) for sulphate functional groups were in good agreement with the reported values [30,33,34].

FTIR absorption bands were also checked by registering spectra on aqueous liquid phase. As depicted in Figure 2B, the FTIR spectrum of a magnesium sulphate standard diluted in water exhibited a well defined strong and broad band, with a maximum at 1110 cm^{-1}. Other identified bands shown on Figure 2A were overlapped by the strong water absorption. Nevertheless, the absorption spectrum showed that the broad and strong IR-absorption band centred at 1110 cm^{-1} could be used for quantitative analysis of sulphate.

Effect of instrumental and experimental conditions

A preliminary assay was conducted -using an aqueous sulphate solution- to determine the moment when the signal had a constant and maximum intensity. As can be seen in Figure 3A, a signal of constant-and-maximum intensity was obtained about 3 min. Therefore, in order to ensure reliable results, sample-to-sample analysis time was set at 3.6 min before recording each spectrum.

The effects of the number of nominal spectral resolution and the number of accumulated scans employed for data acquisition were evaluated. In this sense, the number of accumulated scans per spectrum was varied from 1 to 30, working with a fixed spectral resolution of 4 cm^{-1}, and also the nominal resolution varied from 2 to 16 cm^{-1} averaging 20 scans. As can be seen in Figure 3B, scan variability at fixed nominal resolution had little influence on intensity of the signal, but the time acquisition increased linearly as the number of scans increased. Keeping in mind a compromise with respect to sensitivity, spectral acquisition time and precision, 20 scans were selected, which implicated a measurement time of 1.15 min per spectrum. Under these experimental conditions, the analytical signal was slightly decreasing as the nominal reso-

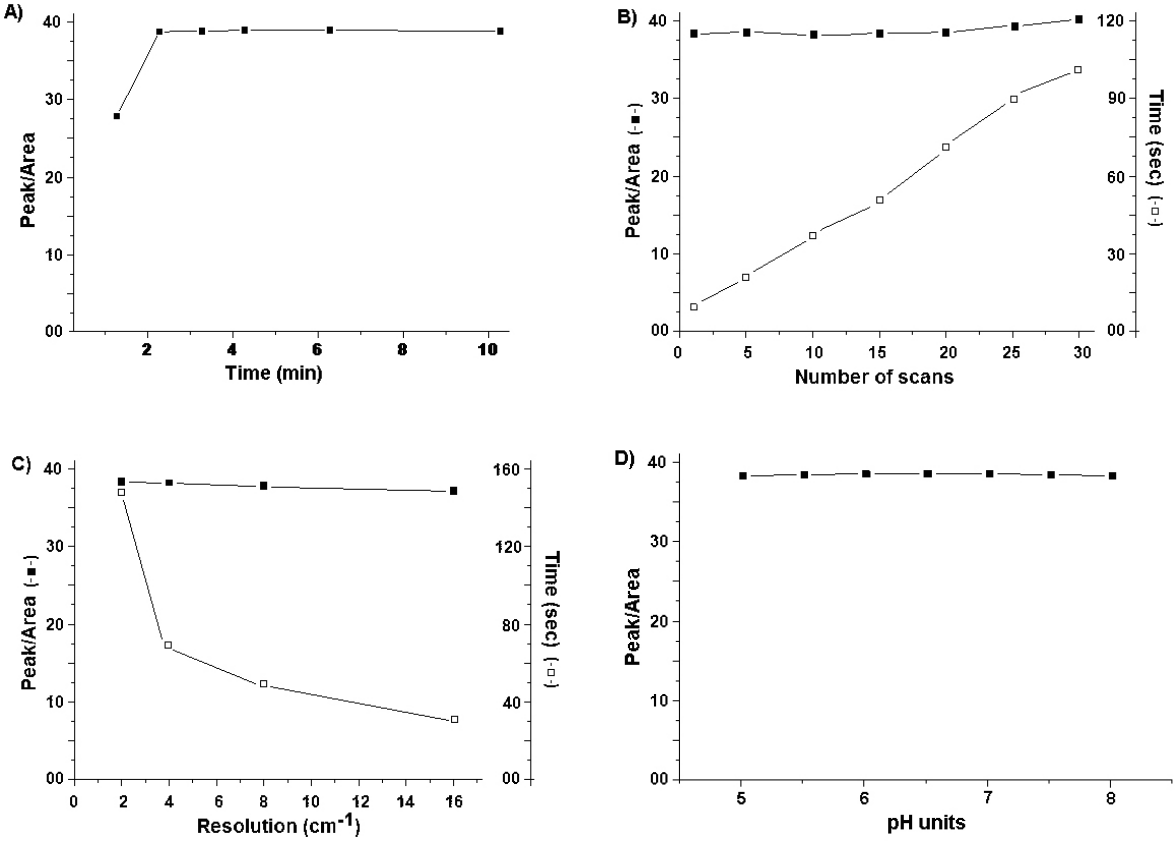

Figure 3. **A**) Effect of increasing analysis times on signal intensity under optimized conditions. **B**) Effect of the number of accumulated scans on signal intensity (■) of a sulphate standard solution using a nominal resolution of 4 cm^{-1}. **C**) Effect of the nominal resolution on signal intensity (■) of a sulphate standard solution using an average of 20 accumulated scans. Acquisition time for each measurement condition is also showed to the right side (❑) of Fig. 3B and 3C. **D**) Effect of changing the pH of a sulphate standard solution on signal intensity. Concentration of the sulphate standard solution corresponds to 25 mg $MgSO_4.7H_2O$ mL^{-1}. Peak area values (n = 3) were recorded with baseline correction between 1185 and 1011 cm^{-1} using water as background.

lution was changed from 2 to 16 cm^{-1} (Fig. 3C). The most intense and precise results were achieved with the lower nominal resolution, but with an acquisition time higher than 2.5 min. By selecting the next nominal resolution (n = 4 cm^{-1}) under study, acquisition time was reduced almost 3 times without affecting relatively the precision. Therefore, in order to ensure a compromise between time-consumption, sensitivity and precision, a nominal resolution of 4 cm^{-1} was selected for further studies.

Since the pH of parenteral pharmaceutical formulations containing magnesium sulphate heptahydrate may need adjustment of pH close to the neutrality, a related assay was conducted. As illustrated in Figure 3D, sulphate FTIR measurements under the optimized conditions were not dependent on pH variability within the range 5.0 to 8.0. In addition, no displacement of the maximum centred at 1110 cm^{-1} was observed as a function of pH variability.

Specificity

To test the specificity, spectrum of each pharmaceutical formulation was registered and compared with the spectrum of the reference substance ($MgSO_4.7H_2O$). A standard solution of the analyte at the expected concentration in the assay procedure and a sample solution with equivalent concentration were prepared. Additionally, another solution was prepared by mixing both of them at an equimolar proportion. Representative spectra of this study are shown in Figure 4A for one of the commercial samples (S1). As can be seen, the individual spectra of the two solutions (standard and sample) and the absorption spectrum of the equimolar solution did not show apparent spectral differences, which was an indicative of absence of matrix interferences from either the excipients or sample preparation. Spectral interferences were not also observed when exploring the first- and second-order derivative spectral behaviour (Fig. 4 B-C).

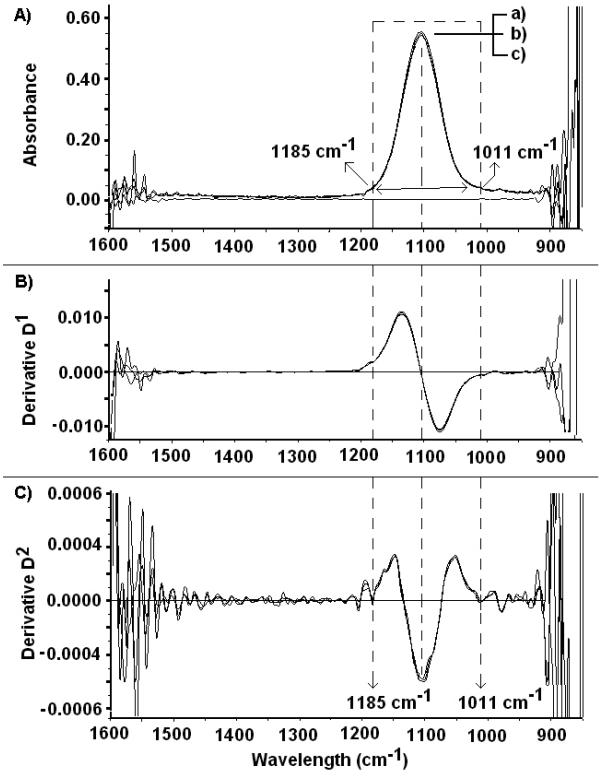

Figure 4. FTIR spectra of magnesium sulphate heptahydrate. **A**) Absorption spectra. **B**) First derivative of the absorption spectra. **C**) Second derivative of the absorption spectra. Concentration of the solutions: a) standard containing 25 mg.ml^{-1}; b) mixture 1:1 of a standard containing 25 mg.ml^{-1} and a sample containing an equivalent concentration (25 mg.ml^{-1}); and c) sample containing an equivalent concentration to 25 mg.ml^{-1}. Spectra were recorded at a nominal resolution of 4 cm^{-1} with 20 co-added scans. The arrows represent the anchorage points for baseline correction.

Identical behaviour was found for the rest of the pharmaceutical formulations (Fig. not shown).

Selection of the measurement conditions

As it was discussed earlier, the IR-spectrum of magnesium sulphate in an aqueous medium only shows one band of possible analytical interest (Fig. 2B). In this way, band height and band area absorbance measurement modes were evaluated -under baseline correction criteria- in order to choose the best analytical performance of the FTIR determination of magnesium sulphate in pharmaceuticals. Baseline correction was firstly established between 1250 an 950 cm^{-1}. However, more precise results were obtained by selecting the interval between 1185 and 1011 cm^{-1} as the baseline correction. This was realized upon inspection of the second-order derivative spectrum (Fig. 4C). This approach eliminated the obvious spectral differences observed in the second order spectra found between the regions 1250-1185 and 1011-950 cm^{-1}.

Owing to the high sulphate concentration declared by parenteral formulations, several calibration curves were prepared. They were obtained from a linear plot of sulphate concentration versus both, IR band height and IR band area of the absorption of sulphate. The measurement criterion was selected taking into account results related to linearity, dynamic range, detection limit, quantification limit and precision (Table 1). Both measurement criteria showed a straight line with acceptable correlation coefficient (> 0.999) within six concentration levels, from 20 to 200% of the expected concentration in the assay procedure (25 mg.ml^{-1} of $MgSO_4.7H_2O$). As expected, it was evident that both measurement criteria showed similar good performance characteristics. However, based on the best features, IR band area measurement with baseline correction was selected for further studies.

Linearity was tested on three different days using the selected working range. No significance differences were found by comparing correlation coefficients, slopes and precision. An excellent correlation coefficient (> 0.999) with a y-intercept less than 0.5% of the response obtained for the analyte at the selected analyte concentration (25.0 mg.ml^{-1}) was obtained (Table 1). The sensitivity and the dynamic range could be considered largely enough to carry out the analysis of the selected pharmaceutical formulations.

Under the selected range, instrumental precision was 0.4% (RSD, n = 10) using a sample solution of concentration equivalent to the middle of the working range. Intra-assay precision, on one day, was carried out using three solutions, each of which was independently prepared according to the stated procedure and analyzed by triplicate. The obtained variation coefficients were 0.8% (RSD) for sample solutions and 1.2, 0.6, and 0.1% (RSD) for standard solutions at the lowest, middle and higher concentration within the working range, respectively. Day-to-day precision, by using a standard solution prepared at the middle concentration of the working range was better than 2.0% (RSD, n = 3).

As can be inferred, the processed data provided acceptable results for achieving the stated objective, i.e. quantitative determination of magnesium sulphate in pharmaceuticals by FA-FTIR.

Parameters	Measurement criterion	
	Band height	**Band area**
Peak maximum/Baseline correction	(1110) 1250–950	(1110) 1185-1011
Working range (mg.ml^{-1})	5.0-50.0	5.0-50.0
External calibration line ($Y = a + b[X]$) [a]	Y = (0.003±0.001) + (0.02568±0.00005) [X]	Y = (0.05 ± 0.05) + (1.960 ± 0.002) [X]
Determination coefficient (r^2)	0.9999	0.9999
Precision (% RSD) [b]	0.6	0.8
Limit of detection (mg.ml^{-1}) [c]	0.33	0.26
Limit of quantification (mg.ml^{-1}) [c]	1.09	0.87
Analytical frequency (samples h^{-1})	12	12

Table 1. Analytical features of the FA-FTIR determination of magnesium sulphate heptahydrate in pharmaceuticals using two measurement criteria with baseline correction. [a] $Y = (a \pm s_a) + (b \pm s_b)[X]$: where "$a$" and "$b$" correspond to the intercept and the slope, respectively, ± their corresponding confidence level at 95 %; being "Y" the signal and [X] the concentration of $MgSO_4.7H_2O$ expressed in mg.ml^{-1}. [b] Relative standard deviation obtained by using three sample solutions, each of which was independently prepared at a concentration equivalent to 25 mg.ml^{-1} and analyzed by triplicate. [c] LOD and LOQ calculated as signal to noise ratio of 3 and 10, respectively, generated by the statistical data of the linear calibration.

			Proposed method	**Reference method**	
Sample	**Pharmaceutical formulation [a]**	**Nominal concentration (%) [b]**	**Found concentration (%) [c]**	**Found concentration (%) [d]**	**Comparison [e]**
S1	Injection	50 (w/v)	47.2 ± 0.2 (w/v)	48.7 ± 0.3 (w/v)	computed t-value = 0.55
S2	Injection	12 (w/v)	11.8 ± 0.5 (w/v)	12.1 ± 0.5 (w/v)	
S3	Injection	12.3 (w/v)	11.8 ± 0.4 (w/v)	12.3 ± 0.3 (w/v)	
S4	Epson salt	100 (w/w)	99.7 ± 0.3 (w/w)	99.3 ± 0.2 (w/w)	
S5	Epson salt	100 (w/w)	99.2 ± 0.8 (w/w)	100.0 ± 0.2 (w/w)	

Table 2. Determination of magnesium sulphate heptahydrate in pharmaceuticals by the reference method (titrimetry) and the proposed method (FA-FTIR). [a] Suppliers of the five analyzed pharmaceuticals are described on the experimental section. [b] Concentration values are expressed as $MgSO_4.7H_2O$. [c] Concentration values are the average of three independent triplicate analyses ± their relative standard deviation in percentage. [d] Concentration values are the average of three replicates of the same sample ± their relative standard deviation in percentage. [e] Two sample t-test to analyze both methods at 95% confidence level, where tabulated t-value = 2.78.

Accuracy and analysis of magnesium sulphate in commercially available pharmaceuticals

In order to finish the validation of the developed FA-FTIR method, five pharmaceuticals containing magnesium sulphate were analyzed by both, the FA-FTIR developed procedure and the titrimetric reference method [5]. A two sample t-test was computed at 95% confidence level to compare the results of the present method with those of the reference method (Table 2). Since computed t-value (0.55) was lesser than tabulated t-value (2.78), there was very little evidence of a significant difference in the average estimates for the two methods. Furthermore, the found concentrations of the five commercial samples were very close to the labelled concentrations.

Despite the fact that the parenteral formulations contain a very simple matrix [35], the technique of standard addition was applied. The calibration set was prepared by addition of different concentrations of standards -within the working range- to a constant concentration of the sample prepared in triplicate. As can be seen in Table 3, there was not evidence of significant differences at 95% confidence level between the slope generated by the external calibration line and the slope generated by the standard addition calibration. This last approach

Sample [a]	Concentration ($mg.ml^{-1}$) [b]			Calibration by external method and standard addition method, respectively [c]	Recovery (%) [d]
	Nominal	Added	Found		
S1	9.86	10.007	20.13	$Y = (0.05\pm0.05) + (1.960\pm0.002)\,[X]$; $r^2 = 0.9999$;	102.6 (±0.9)
		20.014	29.79	($SD_{y/x} = 0.171$)	99.6 (±0.9)
		30.021	40.10	$Y = (19.6\pm0.1) + (1.957\pm0.005)\,[X]$; $r^2 = 0.9999$;	100.7 (±0.2)
		40.028	49.83	($SD_{y/x} = 0.335$)	100.0 (±0.3)
S4	9.93	10.007	19.93	$Y = (0.05\pm0.05) + (1.960\pm0.002)\,[X]$; $r^2 = 0.9999$;	99.9 (±0.2)
		20.014	29.79	($SD_{y/x} = 0.171$)	99.2 (±0.9)
		30.021	39.91	$Y = (19.57\pm0.06) + (1.956\pm0.004)\,[X]$; $r^2 = 0.9999$;	99.9 (±0.2)
		40.028	48.77	($SD_{y/x} = 0.176$)	97.0 (±0.5)

Table 3. Recovery studies of magnesium sulphate heptahydrate in pharmaceuticals by the proposed FA-FTIR method. [a] Identity of the two analyzed pharmaceuticals are described on the experimental section. [b] Concentration values of $MgSO_4.7H_2O$ were calculated using the external calibration line. [c] $Y = (a \pm s_a) + (b \pm s_b)\,[X]$: where "*a*" and "*b*" correspond to the intercept and the slope, respectively ± their corresponding confidence level at 95%; being "*Y*" the signal and [*X*] the concentration of $MgSO_4.7H_2O$ expressed in $mg.ml^{-1}$; r^2 is the determination coefficient and $SD_{y/x}$ is the computed residual standard deviation of the calibration line. [d] Concentration values are the average of three replicates of the same sample ± their relative standard deviation in percentage.

was also used to determine recovery of spiked analyte. The found mean recovery was 100 ± 3% over the working range (40-160% of the target concentration). These complementary studies confirmed once again the accuracy of the proposed methodology.

Interferences from the excipients of parenteral formulations containing magnesium sulphate

According to most of the electronic medicines compendiums, parenteral formulations containing $MgSO_4.7H_2O$ have a very simple matrix. They contain no bacteriostatic agent or other preservatives [35]. May contain sodium hydroxide or sulphuric acid for pH adjustment close to the neutrality [35]. Nevertheless, it is unlikely that the pharmaceutical industry uses sulphuric acid to adjust the pH of these parenteral formulations since aqueous solutions are neutral or slightly acidic; pH 5.9 when diluted to a concentration of 5% (w/v). In the same way, Epsom salts corresponds to pure $MgSO_4.7H_2O$, *i.e.* no excipients are added.

The facts mentioned above were consistent with the obtained FTIR spectra. FTIR spectral inspection of standards spiked with samples did not show excipients interferences (Fig. 4 A-C). Furthermore, the accuracy studies also showed statistically the absence of matrix interferences (Tables 2 and 3). Presence of foreign ions such as OH^-, Cl^-, H^+ and Na^+ (from NaOH and HCl, respectively, used for pH adjustment) did not showed undesirable effects on the determination of sulphate as it was demonstrated in the preliminary studies (Fig. 3D).

On the contrary, excipients containing carbohydrate moiety should interfere in the determination of sulphate because of its absorption in the fingerprint region. Nevertheless, as stated above, excipients which are often used as additives in pharmaceutical products that contain magnesium sulphate are not from this nature.

Robustness of the proposed FA-FTIR method

The proposed FA-FTIR method was very undemanding since once the sample has been prepared by either a simple dilution (parenteral formulations) or dissolution (solid generic formulation), it could be directly analyzed. Neither sample handling nor cell-cleaning handling was further required because of the used continuous-flow mode. This last strategy was also independent from variability of the flow rate. Measurements were unaffected by pH variability at least within 6.5 (±1.5) units. Changes on chemical reagent concentrations were not expected since the only chemical reagent used in the proposed method was just water. The almost total absence of parameters that affect the proposed method was seen reflected in the obtained good accuracy and precision.

CRITICAL OVERVIEW

The developed FA-FTIR method can be used as a quality control tool for rapid authentication of magnesium sulphate in raw material and Epsom salts, apart from quality control of finished products containing magnesium sulphate such as parenteral formulations. Analytical separation techniques are not needed. Samples can be analyzed after either dissolution of solid samples (salts) or simple dilution of those formulated as electrolyte solutions. Water was used as solvent and not other chemical reagents were needed; consequently this strategy meets the requirement of the modern tendency towards Green Analytical Chemistry.

Although the approach could be criticized because it is a determination of magnesium sulphate using the sulphate anion instead of the magnesium cation, there are not reasons to apply the developed method if no other source of sulphate is present in the formulation. In addition, some drug analysts could be interested on analysing other pharmaceutical products containing sulphate as counter-ion using the proposed strategy.

Due to the inherent simplicity of the IR spectrum, the actual interpretation may be indeed easy and the operation requires little experience. What is more, not sophisticated FTIR equipment is required because accepted spectroscopic techniques for pharmaceutical verification -pharmaceutical industry and regulatory agencies- include FTIR spectroscopy.

The method was validated according to ICH guidelines recommendations and fulfilled all requirements. The proposed FA-FTIR method has shown an acceptable sampling rate, versatility, easy of process implementation, minimal sample handling and zero residue production. Sampling rates could be increased by using an appropriate IR flow-cell which tolerates high liquid flow rates instead of the use of a sealed transmission cell adopted for continuous FA.

The proposed method could be adopted for analysing other parenteral formulations containing sulphate as counter-ion such as copper sulphate and zinc sulphate, among others. An additional application that could improve the interest of the method is quality control of sucralphate which is a sucrose sulfate-aluminium complex used for the treatment of peptic ulcers. This product is mentioned because its molecule contains eight sulphate groups ($C_{12}H_{54}Al_{16}O_{75}S_8$).

Acknowledgements. The authors gratefully acknowledge the financial support of the Council of Scientific, Humanistic and Technological Development (CDCHT) of the University of Los Andes (ULA) from Venezuela and the National Fund of Science and Technology (FONACIT) from Venezuela, for providing financial support throughout Projects FA-371-06-08-B and G2005641, respectively.

REFERENCES

1. Drug Information Online (2008) *"Epsom salt medical facts from Drugs.com",* < http://www.drugs.com/mtm/epsom-salt.html>.
2. Spilva A., I. Muktans & R. Navarrete (2008) *"Guía Spilva de las Especialidades Farmacéuticas",* 30th Ed, Ed. Global Editions, Caracas, p. 489.
3. Lumbiganon, P., A.M. Gülmezoglu, G. Piaggio, A. Langer & J. Grimshaw (2007) *B. World Health Organ.* **85**: 763-6.
4. Magnesium Online Library (2008) *"The Magnesium Web Site",* (P. Mason, ed.) < http://www.mgwater.com/index.shtml>.
5. United States Pharmacopeia (2006) *"Magnesium sulphate injection",* 29th Ed, Ed. United States Pharmacopeial Convention, Rockville, MD, p. 1459.
6. Tesfaldet, Z.O., J. van Staden & R.I. Stefan (2004) *Talanta* **64**: 981-8.
7. Idriss, K.A., H. Sedaira & H.M. Ahmed (2001) *Talanta* **54**: 369-75.
8. Yu, B.S., Q.G. Yuan, L.H. Nie & S.Z. Yao (2001) *J. Pharmaceut. Biomed.* **25**: 1027-32.
9. Kallio, M.P. & P.K.G. Manninen (1995) *Anal. Chim. Acta* **314**: 67-75.
10. Gupta, V.K., S. Chandra & R. Mangla (2002) *Sensor Actuat. B-Chem.* **86**: 235-41.
11. Abarca, A., E. Canfranc, I. Sierra & M.L. Marina (2001) *J. Pharmaceut. Biomed.* **25**: 941-5.
12. Dombovari, J., J.S. Becker & H.J. Dietze (2000) *Int. J. Mass. Spectrom.* **202**: 231-40.
13. Kolthoff, I.M., E.J. Meehan, E.B. Sandell & S. Bruckenstein (1969) *"Quantitative Chemical Analysis",* 4th Ed, Ed. Macmillan, New York.
14. Wanessa, R.M. & F.R.P. Rocha (2008) *Anal. Chim. Acta* **616**: 56-2.
15. Reisman, D.J., V. Sundam, S.R. Al-Abed & D. Allen (2007) *Talanta* **71**: 303-11.
16. Xu, Q., C. Xu, Y.P. Wang, W. Zhang, L.T. Jin, K. Tanaka, H. Haraguchi & A. Itoh (2000) *Analyst* **125**: 1799-804.
17. Crnkovic, P.M. & A.O. Jacintho (2002) *Quim. Nova* **25**: 254-8.
18. Buraham, R., K. Higuchi, M. Oshima, K. Grud-

pan & S. Motomizu (2004) *Talanta* **64**: 1147-50.
19. Fung, Y.S., C.C.W. Wong, J.T.S. Choy & K.L. Sze (2008) *Sensor Actuat. B-Chem.* **130**: 551-60.
20. Kuban, P., P. Kuban & V. Kuban (2003) *Electrophoresis* **24**: 1935-43.
21. Sung, H.H., E. Laborde-Kummer, K. Gaudin & J.P. Dubost (2006) *Eur. J. Pharm. Biopharm.* **64**: 33-7.
22. Geiser, L., E. Varesio & J.L. Veuthey (2003) *J. Pharmaceut. Biomed.* **31**: 1059-64.
23. Manley, J.B., P.T. Anastas & B.W. Cue (2008) *J. Clean. Prod.* **16**: 743-50.
24. Gallignani, M. & M.R. Brunetto (2004) *Talanta* **64**: 1127-46.
25. Wartewig, S. & R.H.H. Neubert (2005) *Adv. Drug Deliver. Rev.* **57**: 1144-70.
26. Armenta, S., S. Garrigues & M. Guardia (2007) *Trends Anal. Chem.* **26**: 775-87.
27. Schindler, R. & B. Lendl (1999) *Anal. Commun.* **36**: 123-6.
28. Thanh, H.L. & B. Lendl (2000) *Anal. Chim. Acta* **422**: 63-9.
29. Verma, S.K. & M.K. Deb (2007) *Talanta* **71**: 1546-52.
30. Coates, J. (2000) *"Interpretation of infrared spectra, a practical approach",* in "Encyclopedia of Analytical Chemistry", (R.A. Meyers, ed.) John Wiley & Sons, Chichester, pp. 10815-37.
31. Zhao, L.J., Y.H. Zhang, Z.F. Wei, C. Hua & X.H. Li (2006) *J. phys. Chem. A* **110**: 951-8.
32. Longas, M.O. & K.O. Breitweiser (1991) *Anal. Biochem.* **192**: 193-6.
33. Contreras, C.A., S. Sugita & E. Ramos (2006) *Adv. Technol. Mat.* **8**: 122-9.
34. Dhandapani, M., L. Thyagu, P.A. Prakash, G. Amirthaganesan, M.A. Kandhaswamy & V. Srinivasan (2006) *Cryst. Res. Technol.* **41**: 328-31.
35. RxMed. The Comprehensive Resource for Physicians, Drug and Illness Information (2008) *"Magnesium sulfate",* <http://www.rxmed.com/b.main/b2.pharmaceutical/b2.prescribe.html>.