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Simultaneous Estimation of Mefenamic Acid and Drotaverine HCL in Combined Dosage Form by RP-HPLC Method and Validation of the Developed method

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ABSTRACT

Three new spectrophotometric procedures for the simultaneous determination of Mefenamic acid and Drotaverine HCL are described. The chromatography was performed on an ODS-3V, 250 X 4.6 mm, 5 μ . Column at 25^oC, with a mobile phase Phosphate buffer: Acetonitrile (45:55v/v). The flow rate was 1.5ml/min and UV detection wavelength was 350nm. This method permits the simultaneous determination of Mefenamic Acid and Drotaverine HCL in fermentative foods with detection limits of 5.625 and 0.033 μ g/mL, respectively. The correlation coefficient was found to be 0.998 and 0.999 for Mefenamic acid and Drotaverine HCL respectively. Drug assay was performed in triplicate as a test of accuracy. The average percentage recovery of Mefenamic acid and Drotaverine HCL was found to be 101.2% and 101.1%. The proposed method could be used to be simple, accurate, precise, and rapid and could be used for routine analysis. This condition is applied for tablet dosage form. The statistical parameters and recovery studies were carried out and reported.

Keywords: Mefenamic acid, Drotaverine HCL, Estimation, Tablets, RP-HPLC.

INTRODUCTION

Drotaverine hydrochloride is chemically known as 1-[(3, 4-[diethoxyphenyl) methylene]-6, 7 diethoxy-1, 2, 3, 4 – tetrahydroisoquinoline hydrochloride ^[1]. Drotaverine hydrochloride is highly potent spasmolytic agent ^[2]. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain [3]. Drotaverine hydrochloride is official in Polish Pharmacopoeia [4]. A few UV spectrophotometric [5, 6] and HPLC [7, 8] methods have been reported for estimation of drotaverine hydrochloride. HPLC methods [9]. Mefenamic acid, 2-[(2, 3-dimethyl phenyl) amino] benzoic acid, is an orally active analgesic and anti-inflammatory drug, used to relieve

pain^[10]. Mefenamic acid is official in IP [11], BP [12] and USP [13]. Several UV spectrophotometric [14, 15], HPLC [16-17] and HPTLC [18] methods for the estimation of mefenamic acid have been reported. Literature survey revealed a need for a method capable of simultaneous estimation of drotaverine hydrochloride and mefenamic acid. The objective of this study was to develop and validate a specific, accurate, precise and reproducible quality control method for drotaverine hydrochloride and mefenamic acid in their combination. To our knowledge there is no HPLC method reported for the combination, availability of HPLC method with high sensitivity and selectivity will be very useful for the estimation of Mefenamic acid and Drotaverine HCL in combined pharmaceutical dosage forms. Therefore the aim of the study was to develop a sensitive, precise, accurate and specific HPLC method for the determination of Mefenamic acid and Drotaverine HCL simultaneously in formulation. The present work describes a simple RP-HPLC PDA method for the determination of Mefenamic acid and Drotaverine HCL in tablets. The method was validated according to ICH guidelines and was found to be reproducible with good resolution between Mefenamic acid and Drotaverine HCL.

MATERIALS AND METHODS

Reagents

Mefenamic acid and Drotaverine HCL were obtained as a gift sample from Alkem Laboratories (Mumbai, India) and Curex pharmaceuticals (Jalgaon, India), Sodium Acetate Tri Hydrate (GR-grade) Acetonitrile and Methanol (HPLC grade, MERCK), water (Milli Q). Acetic Acid was procured from Research Lab Fine Chem (Mumbai, India). Other reagents were of AR grade. Mobile phase was filter through a 0.45 μ Millipore nylon 66 membrane filter. Whatman no. 41 filter papers (obtained commercially) were used for the preparation of sample Solutions.

Instrumentation

Quantitative HPLC method was performed on a isocratic HPLC of SHIMADZU 10 AT VP series consisting of LC-10AT liquid pump and SPD 10AVP UV- visible detector. The data acquisition was performed by Spinco Win chrome software.

Chromatographic conditions

The HPLC system consisted of Shimadzu pump LC - 10AT VP and LC-20AD pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spincotech 1.7 software. Analysis was carried out at 350nm using a Inertsil ODS-3V, Reverse phase column of 250x 4.6 mm 5 μ m dimensions at ambient temperature. The mobile phase consisted of Phosphate buffer, Acetonitrile in the ratio of 45:55v/v, filtered through 0.45 μ Nylon that was set at a flow rate of 1.5ml/min.

Preparation of Standard Solutions and Calibration Curve:

The buffer is a mixture of buffer A (0.0019 M citric acid monohydrate and 0.028 M anhydrous Na₂HPO₄) and buffer B (0.05 M KH₂PO₄ and 0.0425 M NaOH) in equal volumes. Buffer and acetonitrile was mixed in the ratio of (45:55), pH was adjusted to 2.5 with ophosphoric acid and the mobile phase was filtered through 0.45 μ m membrane filter (Millipore, USA) and sonicated (Branson sonicator 1510, Germany) prior to use. The mobile phase was used as diluent. Stock solution was prepared by dissolving 200mg of Mefenamic acid, 32mg of Drotaverine HCL working standard were weighed accurately and were transfered in 100ml volumetric flask. 10ml water and 80ml acetonitrile were added sonicated for 15 min and the volume was made up with mobile phase. From the standard stock preparation 5ml of solution was taken in 50ml volumetric flask and further diluted with mobile phase. The solution was filtered through 0.45 μ Nylon membrane filters. A volume of 20 μ L of working standard was injected into column. Calibration

curves were plotted as concentration of drugs versus peak area response. From the standard stock solutions, a mixed standard solution was prepared containing the analytes in the given ratio and injected on to column. The system suitability test was performed from six replicate injections of mixed standard solution.

Preparation of mobile phase

Preparation of phosphate buffer:

6.8 gm of KH_2PO_4 was dissolved in 1000ml of water and adjusted to pH 6 with Potassium Hydroxide then mixed the above buffer.

Mobile phase

Phosphate buffer, Acetonitrile in the ratio of (45:55v/v)

Procedure for Sample Preparation

20 tablets were weighed and average weight was calculated. The tablets were crushed to fine powder. Powder equivalent to 500mg of Mefenamic acid and 80mg Drotaverine HCL was weighed and transferred to 250ml dry volumetric flask was added followed by sonication for 5mins. Then 215 ml acetonitrile was added followed by sonication for 10-15mins. Solution was allowed to cool at room temperature. Volume was made with acetonitrile followed by mixing. The solution was filtered through 0.45μ . Nylon membrane filters. 5ml of above solution was pipetted in 50ml volumetric flask and Volume was made with diluent.

Analysis of Tablet Formulations

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 500mg of Mefenamic acid and 80mg Drotaverine HCL was weighed and dissolved in the 25ml water with the aid of ultrasonication for 10 min and solution was filtered through Whatman paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with the solvent, adding washings to the volumetric flask and volume was made up to mark. The solution was suitably diluted with mobile phase to get $500\mu\text{g mL}^{-1}$ of Mefenamic acid and $80\mu\text{g mL}^{-1}$ of Drotaverine. A typical chromatogram obtained from a sample solution is shown in Fig. 1.

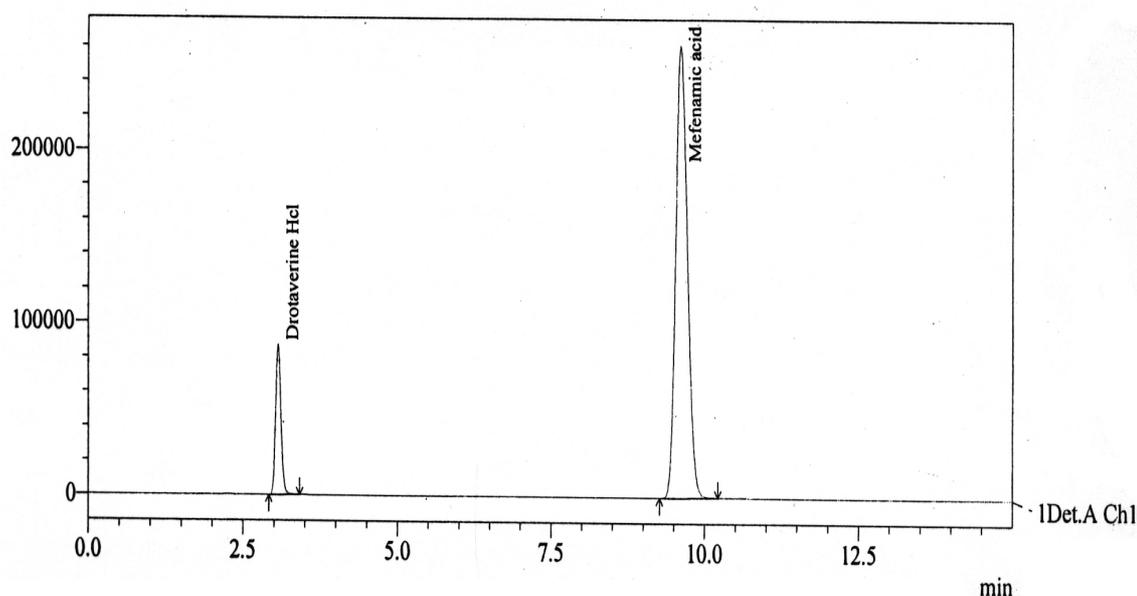


Fig 1. Chromatogram of working standard mixture of $500\mu\text{g mL}^{-1}$ of Mefenamic acid and $80\mu\text{g mL}^{-1}$ of Drotaverine with structure of analytes

Method Development

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines. Assay method precision was determined using nine independent test solutions. The intermediate precision of the assay method was also evaluated as interday and intraday precision. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The mixtures were extracted and analyzed using the developed HPLC method. Linearity test solutions were prepared as described in Formulation analysis. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for analytes were estimated by injecting a series of dilute solutions with known concentration. Values of LOD and LOQ were calculated by using σ (standard Deviation of response) and b (Slope of the calibration curve) and by using equations, $LOD = (3.3 \times \sigma) / b$ and $LOQ = (10 \times \sigma) / b$. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The drug solution was determined using the samples for short-term stability by keeping at room temperature for 12 hrs and then analyzing. The long term stability was determined by storing at 40°C for 30 days. Auto sampler stability was determined by storing the samples for 24 hrs in the auto-sampler. For method development and optimization, retention factor (k) was calculated by using parameters t_R (retention time) and t_M (elution time of the solvent front) and by using the equation

$$k = (t_R - t_M) / t_M.$$

Method Optimization

Well defined symmetrical peaks were obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials that can be summarized. Columns were used for performance investigations, including Inertsil ODS-3V (4.6×250mm, 5 micron) which produced symmetrical peaks with good resolution. The UV detector response of Mefenamic acid and Drotaverine HCL was studied and the best wavelength was found to be 350 nm showing highest sensitivity.

Mobile phase composition

Several modifications in the mobile phase composition were performed in order to study the possibilities of changing the selectivity of the chromatographic system. These modifications included the change of the type and ratio of the organic modifier, the pH, the concentration of acetate buffer, the flow rate, the temperature and the stability of Mefenamic acid and Drotaverine HCL was also studied in methanol and mobile phase. The results obtained are shown in Table 1.

Table 1- System suitability parameters and results of precision at optimized chromatographic conditions

Name of Analyte	System Suitability		Precision of the Method ^b (n=5)		
	Parameter	Value	Actual Conc. (µg mL ⁻¹)	Measured conc. (µgmL ⁻¹), % R.S.D	
				Intra day	Inter day
Mefenamic acid	Theoretical plates ^a	3776	50	50.03, 1.38	59.03, 1.35
	Peak Tailing ^a	1.29	80	79.01, 0.75	80.01, 0.70
	% R.S.D.	0.78	90	86.01, 0.47	90.02, 0.29
Drotaverine HCL	Theoretical plates ^a	8957	100	96.43, 0.93	100.04, 1.76
	USP resolution ^a	3.61	110	106.05, 0.39	110.32, 0.75
	Peak Tailing ^a	1.40	120	114.79, 0.36	120.75, 0.45
	% R.S.D.	0.52	150	148.65, 0.32	150.42, 0.29

^aUSP-NF 29 section 621, pp. 2135. ^bData expressed as mean for "measured concentration" values

Type of organic modifier

Initially acetonitrile and water in different ratios were tried. But in that, both drugs showed peak broadening and the resolution was very less. So Water was replaced by Phosphate buffer, with different pH and concentration. Hence Phosphate buffer: Acetonitrile (45:55v/v) was suitable to get resolved and sharp peak. Methanol was the organic modifier of choice giving symmetrical narrow peaks and good Resolution reported in Table 1.

Ratio of organic modifier

The effect of changing the ratio of organic modifier on the selectivity and retention times of the test solutes was investigated using mobile phases containing concentrations of 60-40% Phosphate buffer. Table 1 shows that 45% Phosphate buffer was the best one giving well resolved peaks and higher no. of theoretical plates. Ratios less than 45% resulted in peaks with very long unacceptable retention times, where as ratios higher than 45% resulted in precipitation in the mobile phase.

Effect of pH

Phosphate buffer did not work at this pH, as the buffer capacity was not effective. The best separation was achieved with acetate buffer 4.5. This is shown in (Fig 2)

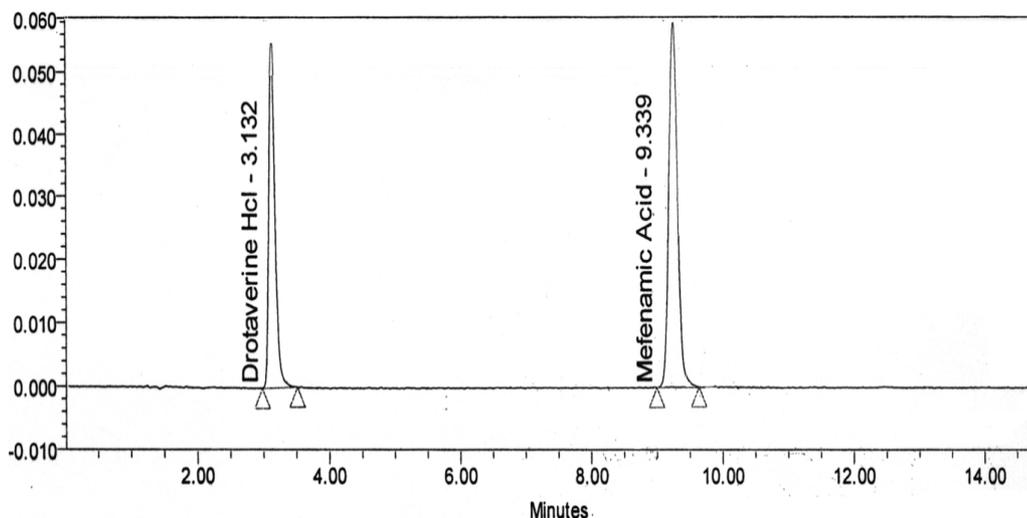


Figure 2: Effect of pH in mobile phase on Mefenamic acid and Drotaverine HCL

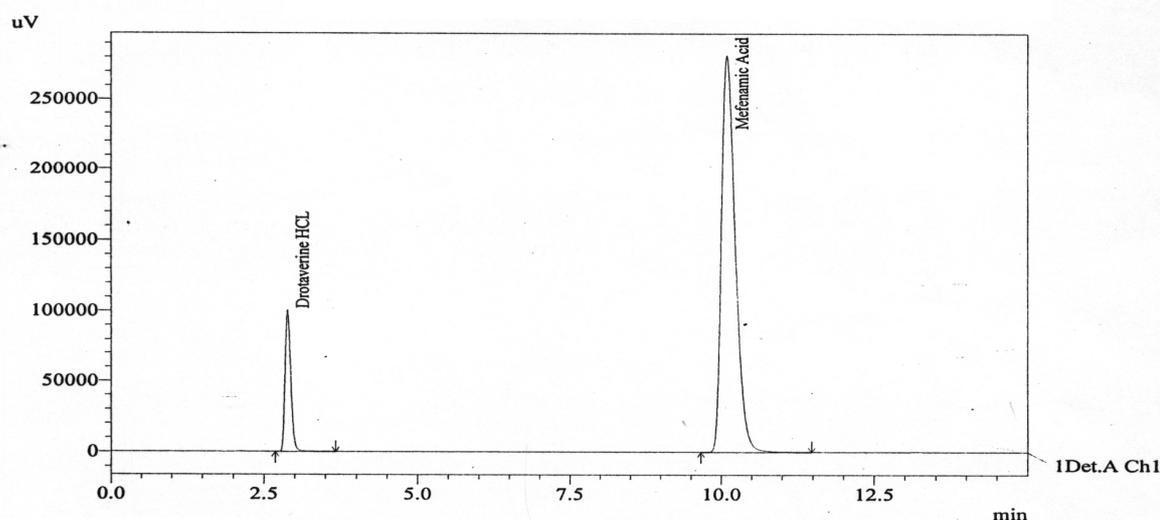


Figure. 2: Effect of Flow Rate on Mefenamic acid and Drotaverine HCL

Effect of Flow rate

The effect of flow rate on the formation and separation of peaks was studied by varying the flow rate from 0.5 - 1.5; a flow rate of 1.5 mL min⁻¹ was optimal for good separation and resolution of peaks in a reasonable time. This is shown in (Fig 2).

RESULTS AND DISCUSSION**Method validation**

The method was validated, in accordance with ICH guidelines, for linearity, range, accuracy, precision, LOD and LOQ, specificity, ruggedness, and robustness^[19]

Linearity and range

For the construction of calibration curves, seven calibration standard solutions were prepared over the concentration range. Linearity was determined for Mefenamic acid and Drotaverine HCL in the range of 50-150 µg mL⁻¹. The correlation coefficient was found to be 0.99998 and 0.99999 for Mefenamic acid and Drotaverine HCL respectively

Precision and Accuracy

The precision of repeatability was studied by replicate (n=5) analysis of tablet solutions. The precision was also studied in terms of intraday changes in peak area of drug solution on the same day and on three different days over a period of one week. The intraday and interday variation was calculated in terms of percentage relative standard deviation and the results are given in Table 1. Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for Mefenamic acid and Drotaverine HCL were 101.2% and 101.1%, respectively, reported in Table 2.

Table 2: Results of Tablet analysis and accuracy studies

Compound (Tablet Label Claim)	Formulation Study (n=6)		Recovery (accuracy) Study	
	Batch Tablet	% Assay Found, % RSD	Recovery Level	% Recovery, % RSD(n=2)
Mefenamic acid	Batch A	101.1, 0.118	50	99.68, 1.034
	Batch B	101.2, 0.122	100	101.2, 1.036
Drotaverine HCL	Batch A	100.2, 0.237	50	100.30, 1.012
	Batch B	101.1, 0.196	100	101.1, 1.015

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ values were found to be 0.12 and 0.36µg mL⁻¹ and 0.15 and 0.45 µg mL⁻¹ for Mefenamic acid and Drotaverine HCL, respectively.

Robustness

Robustness of the method was investigated under a variety of conditions including changes of flow rate, column oven temperature, column form different sup-pliers and wavelength of measurement. The mixed standard solution is injected in three replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust Table 3.

Table .3 Result of robustness study

Factor	Level	Mean % Assay, % R.S.D. of Results	
		Mefenamic acid	Drotaverine HCL
Flow rate (mL min ⁻¹)	1.5	100.08, 0.27	99.99, 0.06
Column oven temperature (°C)	25°C	100.20, 0.45	99.98, 0.24
Measurement Wavelength (nm)	350	99.80, 0.47	100.30, 0.28
Injection volume	20µl	99.80, 0.95	100.04, 0.89
pH	4.5	99.90, 0.78	100.16, 0.06

Specificity

The specificity of the HPLC method is illustrated in Fig. 3, where complete separation of Mefenamic acid and Drotaverine HCL was noticed in presence of tablet placebo. In addition there was no any interference at the retention time of Mefenamic acid and Drotaverine HCL in the chromatogram of tablet solution. In peak purity analysis with photo diode array detector, purity angle was always less than purity threshold for all the analytes. This shows that the peak of analytes was pure and excipients in the formulation did not interfere the analytes.

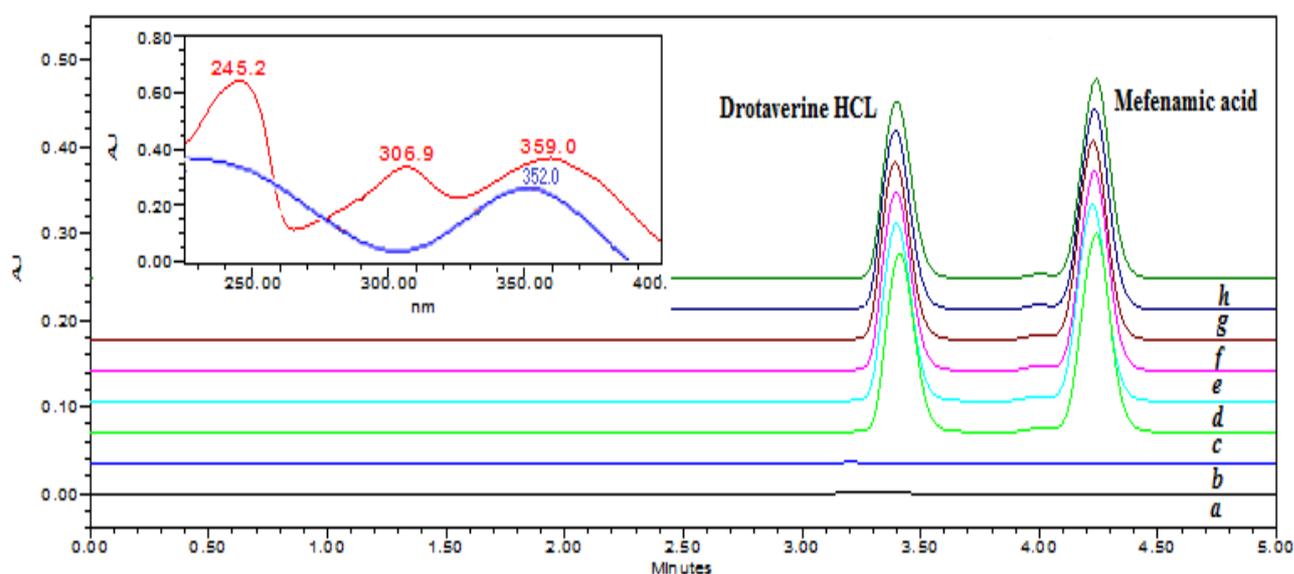


Fig 3: Specificity Chromatogram consists of a) Mobile Phase, b) Placebo, c) formulation, d-h) system suitability standards of Mefenamic acid and Drotaverine HCL (500 µg mL⁻¹) and ACE (80 µg mL⁻¹) and online overlain PDA spectra of analytes

Solution stability studies

Stability as described in method development under experimental section was studied. Result of short term, long-term and the auto sampler stability of the Mefenamic acid and Drotaverine HCL solutions were calculated from nominal concentrations and found concentration. Results of the stability studies were within the acceptable limit (99 –101%).

CONCLUSION

Linear, precise, and accurate RP-HPLC-PDA method has been developed and validated for quantitative determination of Mefenamic acid and Drotaverine HCL from tablet formulations. The manuscript describes, for the first time simultaneous estimation of the combination. All the parameters for the two titled drugs met the criteria of ICH guidelines for method validation. The method is very simple, specific, reliable, rapid and economic nature as all peaks are well

separated and there is no interference by excipients peaks with total runtime of 5 min, which makes it especially suitable for routine quality control analysis work. The method can be extended for determination of analytes in plasma.

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