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COMPARATIVE STUDY FOR DIRECT EVALUATION OF MONTELUKAST SODIUM IN TABLET DOSAGE FORM BY MULTIPLE ANALYTICAL METHODOLOGIES

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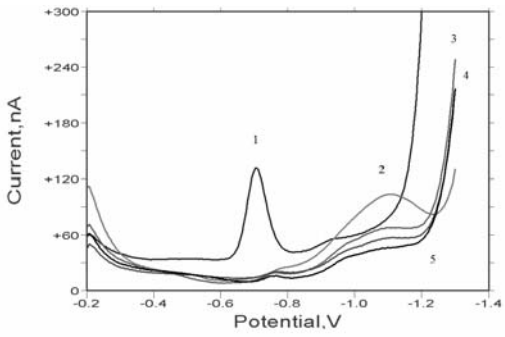
Different non-sophisticated analytical methods consisting of Ultraviolet Spectrometry (UV), Osteryoung Square Wave Voltammetry (OSWV) and flow injection analysis (FIA) were developed, validated according to ICH Q2 (R) 1 guideline and applied for the determination of Montelukast Sodium (MLS) in bulk and tablet formulation. In the UV absorbance spectrum, MLS exhibits more absorption maxima, the main ones being found at the wavelengths of 211.4 and 344.4 nm, respectively. The determination was performed at 344.4 nm. In OSWV determinations rely on the reduction of MLS at the hanging mercury electrode (HMDE). This reduction is mainly diffusion-controlled in Britton–Robinson buffer, a cathodic peak appearing at pH 4.0 in 0.15 M LiCl solutions containing also 40% methanol. In FIA determinations, the carrier solvent was 50% methanol at 1mL /min flow rate. The FIA connected to UV detector. The calibration graphs obtained for the three analytical methods applied in determinations were rectilinear over the ranges of 0.9989, 09976 and 0.9997 for UV, OSWV and FIA respectively. The statistical comparison of the obtained results demonstrates that the three developed methods are very similar in respect of both accuracy and precision.



INTRODUCTION

MLS is selective and orally efficacious competitor of the cysteinyl, CysTL1, leukotriene receptor. It is recommended for the treatment of asthma in kids and grownups.[1, 2] It is the main leukotriene modifier accepted by the US Food and Drug Administration (FDA) in 2008 for use by youngsters from 2 to 12 years old and adults.[3]

The MLS empirical formula is $C_{35}H_{35}ClNNaO_3S$, and its molecular weight is 608.18 g/mol. It is freely soluble in ethanol, methanol, and water and practically insoluble in acetonitrile.[4] It is photosensitive, it becomes unstable and cis-isomer is formed as a photo-degradation product when a solution of MLS is exposed to light.[5] Sulphoxide impurities found at high levels after exposure to oxygen. It deteriorates chemically if the solution remains in contact with atmosphere for a long time. Stability studies of MLS have been reported by capillary electrophoresis and HPLC.[6, 7]

OSWV is considered as a very sensitive and direct analytical technique, which has been used for the rapid and sensitive determination of a wide variety of organic molecules.[8,9] The merits of OSWV over differential pulse voltammetry are the speed of analysis, elevation of the current–response,[10] minor utilization of electroactive compound result in minimizing the chance of electrode obstruction and high sensitivity.[11]

FIA is a modern technique characterized by its utility, simplicity of mechanization, large sampling

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capacity and low sample processing before injection into the system. In recent times, there is a direction to get economical, rapid and green analysis method; FIA techniques fulfill this need in comparison with manual not automatic techniques. Furthermore, in recent years FIA has widespread utilization as they can also be optimized rapidly for the compound analysis in comparison with the conventional chromatographic methods.[12,13]

Spectrophotometric techniques were developed for the determination of MLS in dosage forms[14,15] and in combination with other drugs such as Desloratadine.[16] Several other analytical techniques such as spectrofluorimetric,[17] high performance liquid chromatographic (HPLC) methods[18] were also reported for its determination in human plasma. HPLC and derivative spectrophotometric method for determination of MLS and loratadine in combined tablet was found.[19] Assay of MLS in human serum specimen and MLS tablets on nickel hydroxide nanopetals modified carbon electrode,[20] adsorptive stripping voltammetry of MLS in trade tablets and biological specimen (urine and plasma) using HMDE[21] as well as direct current (DCt), differential pulse polarography (DPP) and alternating current (ACt) polarography for determination of MLS in dosage form and plasma[22] were developed. The superiority of the current developed OSWV method over the traditional electroanalytical methods are no need for special electrode, necessity to accumulate the drug on electrode surface which requires time before detection, highly qualified analyst. In addition, the detection limit of the developed method is considered satisfactory for the determination of MLS in dosage forms.

Overview of the literature showed that there is no reported study for evaluation of MLS in tablet dosage form neither by FIA nor by OSWV is present till now. The main aim of this study is to cope with the demands of pharmaceutical analysis field for reasonable, high-throughput, immediate and sensitive determination of MLS in tablet dosage. The UV method was also developed and recommended for the comparison of OSWV and FIA. These methods would allow determination MLS for routine analysis and quality control in different laboratories equipped with different analytical instruments.

EXPERIMENTAL

1. Materials and Reagents

MLS was supplied from Unimark Remedies Ltd. Batch No.MNT-0330811-B-XJ; other chemicals were of analytical quality acquired from Merck & Co, USA. High purity water was obtained by using a Waters Milli-Q plus distillation system.

2. Instruments

Spectrophotometric estimation was operated on double beam UV-VIS Spectrophotometer Shimadzu 240 version 2.21 utilizing 10 mm quartz cells with 2 nm slit-width.

Polarographic system was Polaropulse Model BAS100B with controlled growth mercury electrode as the working electrode and platinum wire was used as auxiliary electrode and saturated Ag/AgCl was used as a reference electrode. All quantification was done at room temperature 25 ± 2 °C. A pure nitrogen gas 99.999% was used for deoxygenating.

The FIA study was carried out by a Shimadzu (Kyoto, Japan) HPLC system constructed from the LC-20AT pump and a model SPD-20A UV-VIS detector with manual injection of standard solutions and samples. Various instrumental and analytical parameters were examined for each methodology.

3. Standard and sample preparation

The preparation of a stock solution of MLS 163.59 g/mL was done by dissolving the standard in 50% methanol then the working solutions were prepared by further dilution with the same solvent. To protect the solution form degradation by light they were kept in an amber volumetric flask and all experiments were carried out in darkness. The chemical stability of MLS was studied by a voltammetric method for a period of 5 days; the reference solution was stored at 2–8°C in a refrigerator. The voltammetric examination showed a peak signal on the first day only which indicate that the solution is stable for 1 day for this reason working solution were prepared freshly every day.

The method was applied to an MLS tablet according to the United States pharmacopeia rules.[23] For the analysis, 10 tablets were weighed and pulverized. An amount of the powder proportional to 10.0 mg of MLS was weighed precisely and transferred into a 100 mL volumetric flask. After addition of a portion of 50% methanol the mixture sonicated for 30 min, then the volume of the solution was completed to the 100 mL with the same solvent. For UV and FIA methods the aliquots containing MLS were transferred into centrifuge tubes, the solution was centrifuged for 10 min at 20 °C at the speed of 4000 rpm and then filter of 0.45 μm was used for filtration of working solutions. In case of OSWV analysis the solution examined without centrifuge or filtration. The regression equation calculated from the calibration graph of the standard solution was used to calculate the nominal content of the tablets.

RESULTS AND DISCUSSION

1. Ultraviolet Spectrometry

To determine the maximum absorbance of MLS, the standard solution of 12.16 μg/mL was scanned in the range of 200 – 400 nm by 0.1 nm intervals. MLS has many absorption maxims. The complex absorption spectrum of MLS can be explained by the effect of structure and presence of a certain chemical

functional group on UV absorption. Commonly, a molecule has a complex spectrum as it contains more than one chromophore. MLS has conjugated systems that connected with each other. Chloroquinolin connected to the phenyl by the ethenyl group and the phenyl connected to 2-hydroxypropan-2-yl-phenyl by propyl group and to the cyclopropyl acetic acid by thiomethanyl. The presence of saturated and unsaturated functional group between the conjugated systems affects the overall absorption of the conjugated systems. Also, MLS contains C-S-C linkage which gives absorbance at 215 nm. A maximum absorbance at 211.4 can be related to the presence of C-S-C linkage and the shift in absorption is due to the effect of other functional groups of MLS. A maximum absorbance at 344.4 can be related to the presence of the conjugated systems.[24] The peak morphology was good at 344.4 nm. Hence 344.4 nm was observed to be appropriate and efficient for the UV determination further study was done at this wavelength.

2. Voltammetry

2.1. Effect of pH

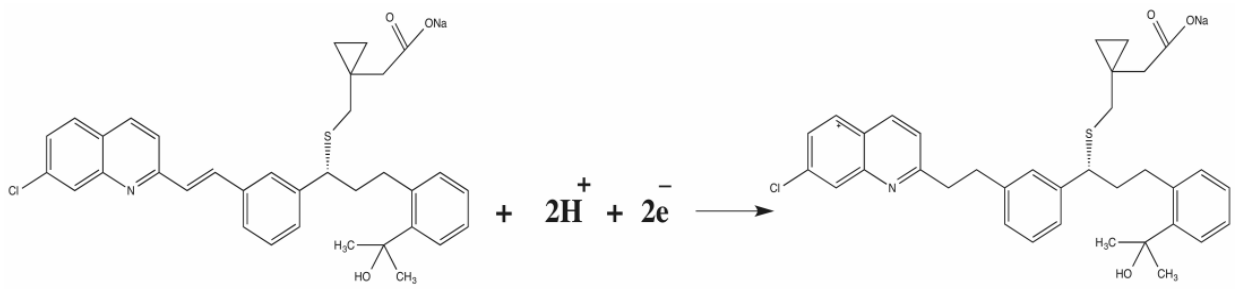
The voltammteric study extremely influenced by the pH of the electrolysis medium because it affects the peak morphology, therefore the effect of pH on the peak potential was fully examined. The voltammogram at the HMDE showed a well-shaped reduction peaks over the pH range of 2.0-12.0. An ill-shaped peak with a negative shift in the potential was observed by increasing the pH especially after pH 8. The negative shift indicates the protonation of the reactive part of the MLS. The reduction occurs at ethenyl group bonded with the quinoline nucleus and the phenyl group as represented in Scheme 1. An ill shape and reduction in the current may be due to adsorption of the MLS according to reference 22. The plot of pH versus the cathodic peak current is stable at pH values from 3.5 to 4.5, which support the reduction process of MLS at the electrode surface. Thus, the solution pH is appropriate to be optimized at 4 in the consequent analytical evaluation.

2.2. Effect of Solvents and Supporting Electrolytes

Supporting electrolyte and solvents are the main component of the examined medium where the electrochemical reaction occurs; they should be inert over the working potentials. For this purpose, the scanning of methanol 20-80%, which is used as a solvent, was carried out, and there was no peak at 20% methanol. The current was at the maximum level at 40% methanol, and the peak morphology was also good at this percentage. The reason of high current and good morphology of the peak at 40% methanol is facilitation of proton transfer between the electrode surface and the solution. Supporting electrolytes lower the resistance of the solution, reduce electro migration effects, and assure a stable ionic strength. The molarity of LiCl 0.05-0.3 M which used as supporting electrolyte was examined, and LiCl concentration of 0.15 M was selected because the current was stable between 0.1 and 0.2 M and in maximum level at 0.15 M of LiCl concentration. Thus, the subsequent analytical determination of the drug was done at these conditions.

2.3. Effect of Instrumental Parameters

Different interrelated instrumental parameters may affect the peak current acquired in OSWV such as amplitude and frequency, but in the current study the instrumental parameters have limited effect on the peak potential. The optimum working conditions such as square wave amplitude 1-200 mV, frequency 1-150 mV, drop size 2-12 and initial potential 0-500 mV was examined. Thus, the instrumental parameters were identified and the subsequent analytical determination of the drug was studied in the following conditions: Square wave amplitude is 25 mV, the frequency is15Hz, drop size 4 and initial potential is 200 mV.



Scheme 1 – Mechanism of electrochemical reduction process for MLS.

3. Flow Injection Analysis

The optimum conditions of FIA were determined for a standard solution of MLS at the concentration of 12.16 µg/mL. MLS is soluble in methanol and insoluble in acetonitril. Therefore, different percentage of Methanol-water 10-100%, v/v was studied as mobile phase. The peak shape was perfect at 50%, v/v concentration of methanol the following determination was performed at this percentage. Different flow rates over the range 0.2-3.0 mL /min was investigated. The flow rate of 1 mL/min was optimally in accordance to the peak morphology.

4. Stability study

In the stability study of MLS by OSWV the compound showed a reduction peak at - 0.7 V on the first day due to reduction of the ethenyl group as mentioned before. But in second day the drug produced small peak at -0.7 V reduction potential which can be explained by the conversion of trans-isomer to the cis form due to exposure to light since it is photosensitive compound as mentioned before, so solution contain only a small percentage of trans–isomer.

A hump shaped peak at -1.1 V was appeared which could be due to reduction of cis-isomer on the mercury electrode at more negative potential. The reason for the negative shift could be explained by considerable variation in electrochemical behavior of isomers and geometric orientation of cis-isomer with the electrode.[25] In the three following days the voltammogram shows only a small peak of the trans-isomer and the peak for reduction of cis-isomer is vanished, which may be due to further degradation of the compound. The absence of peak for the degraded compound could be explained that they are not faradic. The stability study voltammogram is shown in Fig. 1.

5. Analytical performance

ICH Q2 (R) 1 guideline for validation of analytical procedures was applied to evaluate the developed methods.[26] For the three methods, a series of working solution in the range of 3-16.5 µg/mL was prepared by transferring a proper portion of the standard solution 163.59 mg/mL to the volumetric flask and diluting with the 50% methanol. The calibration graphs for the three methods are illustrated in Figs. 2, 3, 4.

The investigation of method linearity was performed with 7 concentrations in the range of (0.5, 1.5, 3.27, 6.5, 9.8, 13 and 16.5µg/mL). Calibration plots were chosen according to the limit of quantification in this range (3.27, 6.5, 9.8, 13 and 16.5 µg/mL) and 3 set were examined by the three techniques. The calibration graph was set up by applying the measured absorbance, signal area and current versus concentration of UV, OSWV, and the FIA. Various statistical parameters for linear regression equation like slope, intercept and sum of square of regression have been calculated respectively and the linear regression equation was calculated. LOD and LOQ were calculated as [(standard deviation of regression equation) / (slope of the regression equation)] by multiplying by 3.3 and 10, respectively.

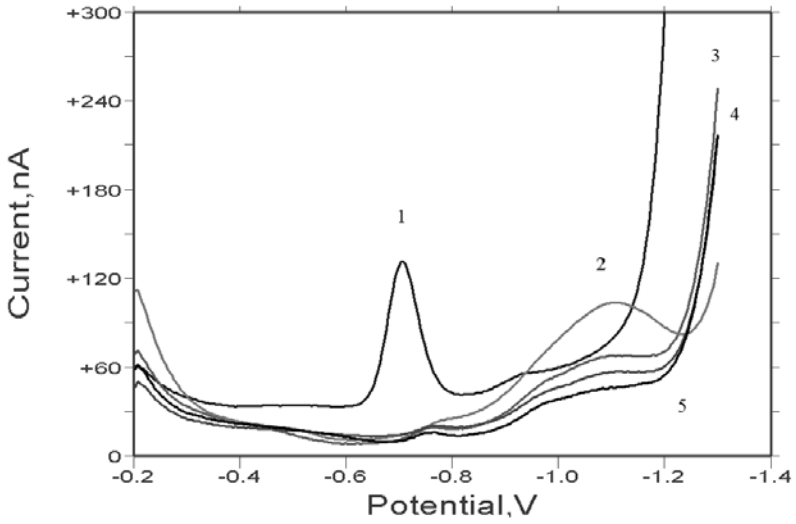


Fig. 1 – OSWV voltammogram of (12.16 µg/mL) for 5 days.

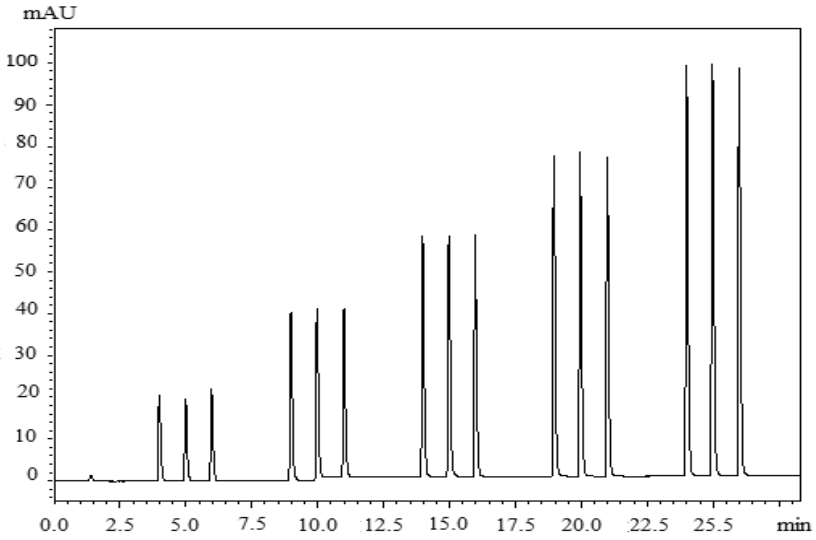


Fig. 2 – Calibration curve **of** MLS by FIA.

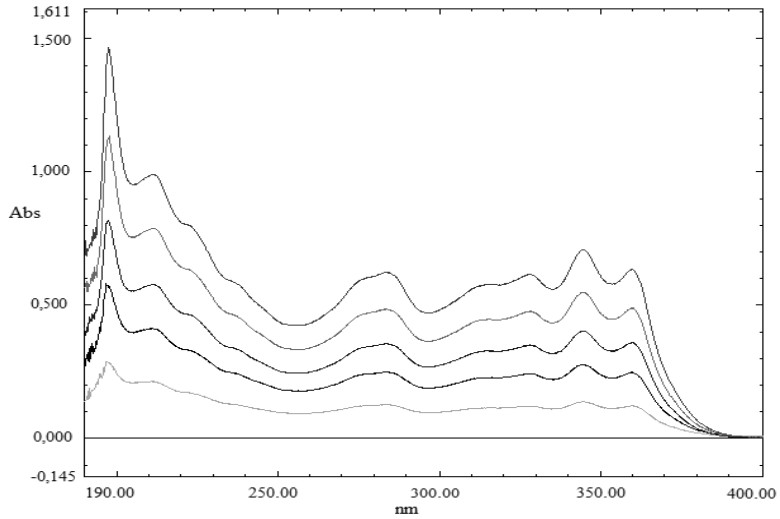


Fig. 3. Calibration curve of MLS by UV.

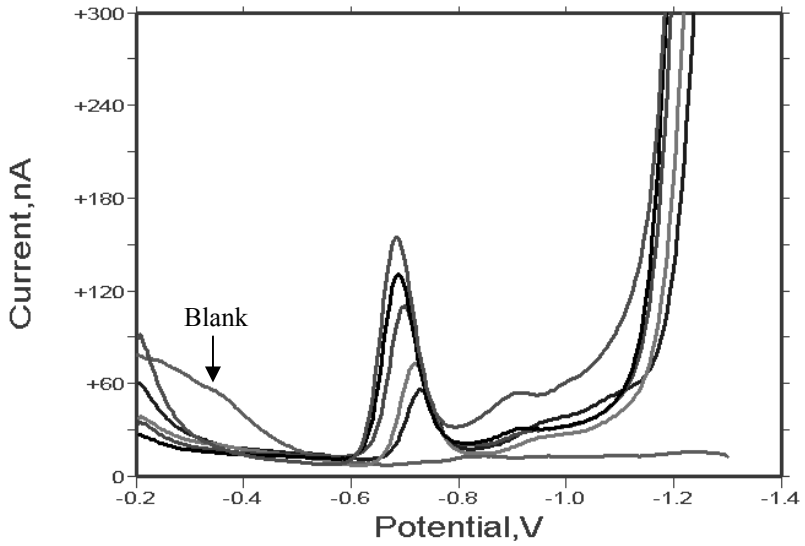


Fig. 4 – Calibration curve of MLS by OSWV.

Table 1

Regression data of the calibration curve for quantitative determination of MLS

Parameters	Analytical Methods		
	Voltammetry	FIA	UV Spectrometry
Measured potential (*V*) and Measured wavelength (nm)	0.6-0.8	344.4	344.4
Linearity Range (µg/mL)	3-16.5	3-16.5	3-16.5
Slope	$6.79x10^{-9}$	34501	0.0429
Intercept	$4.75x10^{-8}$	15189	0.0167
Regression	0.9976	0.9997	0.9989
SD of slope	$1.29x10^{-10}$	514.3	0.00099
SD of intercept	$1.4x10^{-9}$	5587	0.0108
LOD, µg/mL	1.142	0.8881	0.6384
LOQ, µg/mL	3.461	2.691	1.935
Within-day precision (RSD), %	1.788	1.679	1.288
Between-days precision (RSD), %	1.986	1.848	1.467
Accuracy recovery %	Max. 97.96% Med.100.82% Low. 98.02%	Max.100.08% Med.100.24% Low. 98.86%	Max. 101.45% Med. 99.55% Low. 101.52%

SD:Standard Deviation, LOD:Limit of Detection, LOQ:Limit of Quantification, RSD: Relative Standard Deviation

MLS solutions of 3.27µg/mL, 9.8µg/mL, and 16.5µg/mL were used for accuracy studies. The examination of intraday and interday accuracy of the three methods was performed for three subsequent days.[26] The accuracy results are highly acceptable for the determination of MLS since the recoveries percent were almost close to 100% for both drug standard and product.

To examine the repeatability of the method (Intraday) and intermediate precision (Interday), medium concentration 9.8 µg/mL of the MLS standard solution was examined for three days, six times in a day. Good correlations were obtained for both intra and interday's experiments indicating that the developed methods are highly precise and analytically acceptable. The calculated relative standard deviation (RSD) value is lower than 2% deviation from the nominal value of precision.[27] The validation data are given in Table 1.

6. Comparison of the Determination Methods

On the basis of the results in Table 1, the three methods can be accepted as an analytical technique for analysis of MLS in tablet dosage form. By comparing the precision and accuracy of three methods, all of the three methods are adequate from an analytical perspective. From the point of detection limit, UV analysis showed some superiority so it can be applied for the determination of low concentration samples with no need for derivatization steps. In the OSWV determination can be performed without any separation step because the peak obtained from the dosage form was similar to those obtained from the MLS standard with no interference from tablet excipients. Both OSWV and UV analysis are convenient and accurate to be applied in laboratories lacking liquid chromatographic instruments. The uniformity of the mobile solvent in the FIA makes the method considerable in comparison with the customary chromatographic methods. A slight superiority of FIA over the other two methods was seen since, it is suitable for processing numerous samples on a daily basis due to short analysis time, large sample capacity and low solvent consumption especially in quality control laboratories. To prove the developed methods applicability the replicate analysis of pharmaceutical dosage form was performed and the results were evaluated and validated statistically in the Table 2.

Statistical comparison of the developed methods was performed at the 95% confidence level with the assist of Student's t and F-Tests. The obtained results from the analysis of MLS in tablet dosage form by OSWV and FIA are in good agreement with those obtained by the UV methods which is used as a reference method. On the basis of results shown in Table 2, there was no considerable variation between the performances of the three methods since the corresponding theoretical values for Student's t and F-Test were lower than the calculated values.[28]

Table 2

The results for the determination of MLS of dosage form

Parameter	OSWV	FIA	UV Spectroscopy
Labeled claim, mg	10	10	10
Amount found, mg	10.13	10.09	9.97
RSD %	1.851	1.732	1.303
Bias %	-1.3	-0.9	0.3
t-value	2.09	1.63	T-theoretical: 2.57
F-test	2.018	1.76	5.05

CONCLUSION

Three resourceful and uncomplicated methods have been established, optimized and validated for the determination of MLS in tablet dosage form. The OSWV analysis showed simplicity over the other methods due to absence of matrix effect so tablet pretreatment was not required, alongside the determination of MLS in dosage form, stability study of MLS was carried out for the first time by using OSWV. The study gave an idea about the difference in the electrochemical behavior between the *trans* and *cis*-isomer of MLS at mercury electrode. The UV-Spectrophotometry can be used for simple and sensitive determination because of low detection limit. The advantages of FIA over the other two methods are the reduction of analysis time and solvent consumption so it can be applied in high duty laboratories. The analyst can select the method according to the facilities and instruments, which are available in hand for the routine and the quality control of the MLS in the dosage form.

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