Citation: Hasan N, Siddiqui FA, Afridi NS, Chaiharn M, Khan S, et al. (2012) A New Acetonitrile-Free, Cost-Effective, Simple and Validated Rp-HPLC Method for Determination of Montelukast Sodium in Bulk, Tablets and Liquid Dosage Forms. 1: 261. doi: VFLHQWL26FUHSRUV

by passage through RO plant (Waterman, Pakistan) and was further ltered through a 0.45 µm membrane lter (Millipore, Bedford, MA, USA).

Chromatographic conditions

e HPLC analysis was carried out at ambient temperature. e compound was chromatogrphed isocratically with a mobile phase consisting of Methanol (HPLC grade): deionized water (90:10, % v/v) with the pH adjusted to 2.8 ± 0.1 using phosphoric acid. e mobile phase was ltered by passing through a 0.45 µm membrane lter (Millipore, Bedford, MA, USA). e ow rate was 1.0 ml/min, and the injected volume was 20 µL. e e uent was monitored spectrophotometrically at a wavelength of 220 nm.

Apparatus

For chromatography we used a SIL 10A auto injector HPLC system comprising of SCL 10A system controller, SPD 20A prominence UV/VIS detector, with a Shimadzu LC 20 AT pump with LC Solutions so ware. Separation was performed on a Hyperpack ODS C18 HPLC column, (4.6×250 mm; 5 µm bead size) maintained at ambient temperature 25 °C, Ultrasonic cleaner (Elmasoni E 60 H), Jenway 3240 pH meter and Sartorious TE2145 analytical balance. roughout the work only amber glass asks were used to avoid light e ect on the solution of montelukast standards and samples.

Analytical Procedure

Standard preparation

In a 100 ml volumetric ask, weighed accurately about 20.8 mg of Montelukast sodium reference standard. Dissolve up to 50 ml in Methanol (HPLC Grade) sonicate for 10 minutes let it cool to room temperature and make up volume with the extraction solvent stir well for 20 minutes and diluted 2.5 ml in a 50 ml volumetric ask to get 10 µg/ml working standard solution of Montelukast base. Filter through 0.45 micron lter paper. is solution can be used for 3 days, if stored protected from light

Sample preparation

Analysis of tablets: For making sample of 10 µg/ml Montelukast, 20 tablets were weighed and ground to get an evenly homogenized powder. e sample was weighed accurately equivalent to 10 mg of Montelukast and taken in 100 ml volumetric ask and 50 ml of extraction solvent was added. e sample was sonicated for 10 minutes and the placed for stirring for 10 minutes to cool down the temperature and then added extraction solvent up to the mark. e solution was diluted in a 50 ml volumetric ask to get 10 µg/ml working standard solution by adding 5.0 ml of stock solution. e sample was then ltered through 0.45 mm lter paper and injected into the HPLC system.

Analysis of suspension: To prepare a sample of 10 µg/ml Montelukast from suspension, the suspension was shaken well before and was accurately weighed as 11.1gram (density, 1.1 g/ml) equivalent to 10 mg of Montelukast. e sample was taken in 100 ml volumetric ask and 50 ml of extraction solvent was added. e sample was sonicated for 20 minutes and then placed for stirring for 30 minutes to cool down the temperature and then added extraction solvent up to

6(r)-6(in)8(g)-108(f)9(o)1an <</MCID 224 BDC T9(c)6(k)11(sji6-23(t)6(em)5t)1

evaluated using a Symmetry C

18

Method Validation

kt4(e)-6(h)4(o)-9(d)-195(va)-5(lid)-3(a)19(t)-5(io)12(n)-195(wa)3(s)-195g feriBDC /

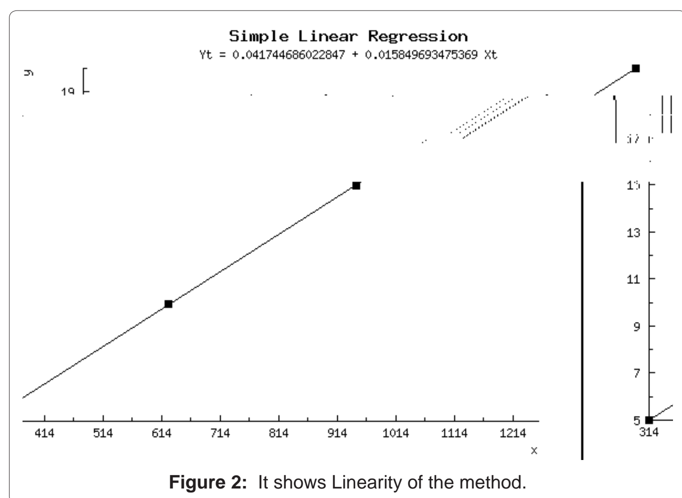

Figure 2: It shows Linearity of the method.

decreasing concentrations, in the range of 10-1.25 ng /ml of Montelukast and injected onto the chromatograph.

Robustness

e robustness was studied by analyzing the same samples of MKT by deliberate variation in the method parameters. Doing small changes in the chromatographic conditions like mobile phase, ow rate etc. and change in the responses of MKT was noted. For this purpose changing in the extraction time of MKT from dosage forms by ± 2 min, composition of mobile phase by ± 2 % of methanol, ow rate by ± 0.2 ml/min and column temperature by ± 2 °C was performed.

System-Suitability

System suitability of the method was evaluated by analyzing the symmetry of the standard peaks, theoretical plates of the column. For this purpose ve consecutive replicate analysis of the drug were assessed in order to investigate the suitability parameters including repeatability, peaks symmetry, and column e ciency (theoretical plates).

Results and Discussion

e HPLC method development for the determination of drugs has received a substantial consideration in the new era of technology, because of their importance in the quality control of drugs and drug products. e major purpose of developing this LC method was to attain determination of the drug in di erent pharmaceutical formulations under economical conditions that are applicable for routine quality control and research & development laboratories.

A number of methods are available for MKT determination [8-18], but many of them are used for certain speci c purposes and no one can be generalized for MKT determination in its di erent forms of pharmaceutical dosages. e literature survey also revealed that almost all the methods developed so far have utilized acetonitrile as a major component in mobile phases [20], due to the supreme solubilizing properties and UV absorbance characteristics of acetonitrile, and there is no counterpart substitute for acetonitrile in the reverse-phase HPLC, UV application. However, keeping in view the increasing shortage of acetonitrile "Great Acetonitrile Shortage", and high cost, laboratories are in search of cost-e ective solutions to manage the impact on their research and business timelines. Also considering the chromatography type and the detection wavelengths in use, it may be possible to replace acetonitrile with methanol or with a longer chain alcohol. Also as Methanol is less expensive than acetonitrile and TFA or TCA etc., therefore the use of methanol as an alternative solvent to acetonitrile was evaluated in MKT analysis on large industrial basis, and a very simple and easy to use method has been developed.

Validation of Method

Linearity

e linearity ranges were found in the range of 5-20 µg/ml. e assay was judged to be linear as the correlation coe cient was greater than 0.995 by the least-square method. A linear correlation was found between the peak areas and the concentrations of Montelukast, in the assayed range. e regression analysis data are presented in Table 1 and Figure 2.

Specificity

Chromatogram shown in Figure 3, proves speci city or selectivity of the assayed method, as chromatogram of Montelukast in samples were found identical with standard chromatogram and no interference peak was observed in sample chromatogram, Peak purities higher than 98.0% were obtained in the chromatograms of sample solutions, demonstrating that other compounds did not co-elute with the main peaks (Figure 3). e chromatogram obtained with the mixture of the tablet excipients proves that here is no any interference from excipient and peak of interest ful ll all the requirements of symmetrical peak, and hence the speci city is con rmed.

Precision

e precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. Intra-day precision of the method was evaluated for montelukast at three di erent independent concentrations i.e. 8, 10, and 12 µg/ml (n=/3) by determining their assay. e RSD values ranged from 0.54 to 1.14% (Table 2) while Coe cient of variation (CV) of the assay results was NMT 3. Inter-day

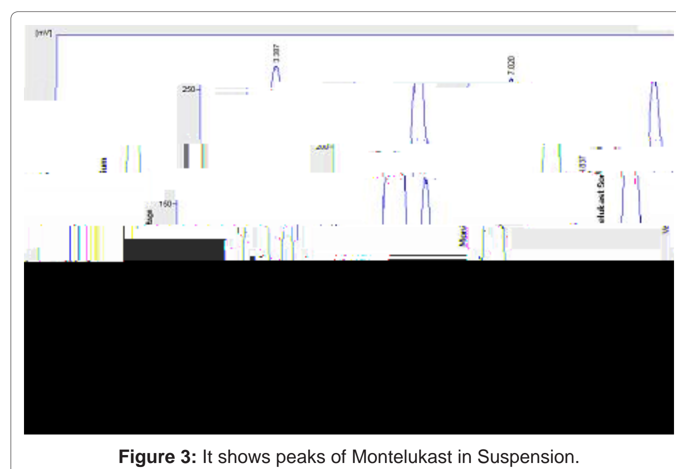

Figure 3: It shows peaks of Montelukast in Suspension.

Nominal concentration J PO	Day 1			Day 2			Day 3		
	Mean	SD	%RSD	Mean	SD	%RSD	Mean	SD	%RSD
8	7.95	0.15	0.81	7.89	0.14	0.87	7.86	0.11	1.14
10	10.03	0.64	0.57	9.98	0.58	0.97	9.91	0.84	1.10
12	12.09	0.77	0.54	11.81	0.67	0.55	12.05	0.67	0.55

Table 2: Inter-Day and Intra-Day Precision of Montelukast (n = 3).

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