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PHARMACOKINETIC EVALUATION OF ONCE-DAILY SUSTAINED RELEASE DEXTROMETHORPHAN HYDROBROMIDE (DM) AND IMMEDIATE RELEASE DM IN HEALTHY HUMAN VOLUNTEERS

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ABSTRACT

The pharmacokinetic study of a newly developed sustained-release dextromethorphan hydrobromide tablet was studied in six healthy human volunteers after single oral administration and compared with an immediate release tablets (IR) in randomized three-period crossover design. A sensitive and rapid HPLC method was developed and validated for the quantitative determination of dextromethorphan hydrobromide in human plasma. The compound and the internal standard (I.S.) (Losartan Hydrochloride) were extracted from the plasma samples by solid phase extraction. The extracts were analyzed by a reversed-phase HPLC with 0.5% triflouro acetic acid: acetonitrile (45:55, v/v) as the mobile phase. The calibration curves were linear in the range between 110.00 to 3000.00 ng/mL. The overall precision (expressed as R.S.D.) of quality controls were within 15%. The method was successfully applied to the pharmacokinetic study of dextromethorphan hydrobromide in the three formulations. The C_{max} of sustained-release tablet (SR) was significantly lower than that of the IR and the T_{max} was significantly longer than that of the IR.

KEYWORDS: HPLC; Dextromethorphan sustained release tablet; pharmacokinetics; healthy volunteers.

INTRODUCTION

Dextromethorphan (DM), the d-isomer of the opiate agonist Levorphanol, is a highly effective and widely used antitussive drug. This drug has been used from 1960s and it has been in use for more than 40 years.[1-2] It is a non-competitive receptor antagonist of the N-methyl- d-aspartate (NMDA) type of excitatory amino acid receptors that are involved in neuronal development and migration. The usual doses of dextromethorphan are 10–20 mg every 4 h or 30 mg every 6–8 h with a maximum of 120 mg daily. It is well-known non-narcotic antitussive agent, is generally used as an ingredient in cough and cold remedies. The dosage of the drug is usually three to four times a day because of its short half-life.[3] Therefore, sustained release dosage forms were developed to avoid repeated administrations and increase patient compliance. Sustained release (SR) delivery systems for oral dosing are effective in achieving optimal therapy with drugs that have a narrow therapeutic range of blood concentration or eliminate rapidly. SR products are designed to bring the blood level of a drug immediately to therapeutic concentrations by means of an initial dose portion and then sustain this level for a certain pre determined time with the maintenance portion.[4-6] At present there are no sustained release dextromethorphan matrix tablets available in India. A sustained release dextromethorphan tablet can lead to the reduction of the number of doses administered; less of a chance of overdose, and it is good dosage form for asthma patients in the night time cough. Several methods have been reported for quantification of DM in biological fluids.[7-17]

In this paper we describe a simple, rapid, sensitive and specific method was developed for the pharmacokinetics study of dextromethorphan in the newly-developed sustained-release tablet (SR). HPLC with UV detection was employed. Compared with the published HPLC method described above, we have developed a sensitive procedure. The validation of the HPLC method, the time-profile of plasma concentrations of dextromethorphan hydrobromide in human and the pharmacokinetics parameters of the SR were evaluated and compared with those of regular immediate-release formulation.

EXPERIMENTAL

Reagents and Chemicals

Dextromethorphan Hydrobromide (DM) (purity 99.8%) was kindly provided by Divi`s laboratories Hyderabad, India. The internal standard Losartan potassium (LP) was purchased from the Cadila pharmaceuticals, Gujarat, India. HPLC-grade acetonitrile was provided by

Merck (Germany). Trifluroacetic acid analytical grade chemicals from S.D.fine chemicals, Mumbai. Purified water from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout. Pharmaceutical products used in the pharmacokinetic study were DM (tablets containing 15 mg DM produced by Roche Pharma) as the reference preparation (immediate Release), and DM (tablets containing 60 mg DM produced by In-house as the test preparations (Slow and fast sustained release).

Instrumentation and Chromatographic Conditions

Analysis was performed on a Shimadzu Scientific Instruments (Kyoto, Japan) liquid chromatographic system composed of a LC-10ADVP pump, a SPD-10ADVP variable wavelength detector, a CTO-10ADVP column oven and a SIL-10ADVP auto-sample injector. The analytical column employed was a VYDAC Monomeric C18 reversed-phase column (250 × 4.6 mm i.d., 5 µm particle size). The mobile phase consisted of 0.5% trifluoroacetic acid: acetonitrile (45:55, v/v). The analyses were conducted at 40°C, flow rate 1.0 mL/min and detection wavelength 280 nm.

Preparation of Standards

Stock solutions and DM and LP (internal standard, IS) were prepared with acetonitrile solution to a final concentration of 1.0 mg/mL and stored at −20°C. A set of eight non-zero calibration standards, ranging from 110.00 to 3000.00 ng/mL was prepared by spiking blank drug free human plasma with an appropriate amount of DM. The quality control (QC) samples at four concentration levels [110 ng/mL (LLOQ), 590 ng/mL (low), 1400 ng/mL (medium) and 3000 ng/mL (high)] were prepared in a similar manner to the calibration standards. Blank human plasma was tested before spiking to ensure that no endogenous interference was found around retention times of DM and IS.

Preparation for Plasma Samples

Calibration standards, validation QC samples and healthy volunteer plasma samples were prepared by adding 0.5 mL plasma to Eppendorf tube followed by adding 10.0 µL internal standard solution (100.0 µg/mL). All samples were mixed by vortexer for 30 s. After these procedures, Samprep SPE Columns C18 (50µm, 70A) 100mg/1mL solid phase extraction cartridge was conditioned with methanol, water sequentially. To this load the above sample. The cartridge was washed with 2.0 ml of water. The drug and internal standard was eluted from the cartridge using 0.5ml of mobile phase. The resulting solution used for the analysis.

Method Validation

A calibration curve (n = 8) and QC samples were prepared by spiking blank human plasma with standard solution of DM. The low limit of quantification (LLOQ) was the smallest analytical concentration of dextromethorphan with a coefficient of variation of less than 20%. The specificity of the method was verified by using six different blank plasma samples obtained from healthy volunteers not taking dextromethorphan tablet. These samples were prepared according to the sample procedure except for the addition of the dextromethorphan and IS. To evaluate the inter-assay accuracy and precision, validation QC samples at concentrations of 110.00, 590.00, 1400.00, and 3000.00 ng/mL were analyzed together with one independent calibration standard curve for three consecutive days, while intra-assay accuracy and precision were evaluated in the same concentration QC samples in replicates of five on the same day. The analytical recovery was calculated by comparing chromatographic area ratios of extracted samples and unextracted standard samples at three different concentrations (590.00, 1400.00 and 3000.00 ng/mL; n = 5) after the volume correction of extracted samples.

Stability

It is necessary for a reliable method to exploit the stability of analytes during analysis time and also upon storage for a limited time. The stability of dextromethorphan hydrobromide was assessed by placing QC samples of three concentration levels at room temperature for 24 h. The freeze– thaw stabilities of dextromethorphan hydrobromide were also evaluated by analyzing QC samples undergoing four freeze thaw cycles at ±20°C. The freeze stability of analyte was estimated by placing QC samples at ±20°C for one month. The samples were brought under room temperature to thaw and measured after 30 days.

Pharmacokinetic study

Six healthy male volunteers (aged 18–30 years) were included in the study, after having undergone a thorough medical examination. The Bioavailability was performed in accordance with the guidelines set by the World Medical Assembly (Declaration of Helsinki). All volunteers gave written informed consent to participation in the study, after having been informed of the nature and implications of the study. A total of 6 male healthy subjects completed this study. There were no dropouts. Their mean age was 24.8±3.8 years (range 21–30 years), their mean weight was 72.4±7.3 kg (range 64–84 kg) and their mean height was 180.6±6.7 cm (range 167–192 cm). All cardiovascular measurements and laboratory values at

screening were within prescribed limits. The study was an open, randomized, three-period, three-group crossover trial with an 7-day washout interval. During the first period, volunteers from group A received a single 15 mg dose of immediate release Dextromethorphan tablets from ROCHE PHARMA, S.A (Reference, Romilar), while volunteers from group B received a single 60 mg dose of slow sustained-release tablets (Test, In house, India). While volunteers from group C received a single 60 mg dose of fast sustained-release tablets (Test, In house, India). During the second and third period, the procedure was repeated on the groups in reverse. The tablets were administered to the volunteers in the next morning after an overnight fast, with 250 ml of water. Volunteers received standard lunch and supper, respectively, 4 and 10 h after drug administration. Volunteers did not ingest any alcoholic drink coffee or other xanthine-containing drinks during the trial. Furthermore, they did not take any other drug, 2 weeks before the study and during the execution. Blood samples were taken at pre-dose (0 h) and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0 and 24 h. During each hospitalization period, the subjects remained under constant medical surveillance by a physician. During the three study sessions, the subjects maintained daily contact with the clinical investigator and reported any adverse events, whether related or not to the ongoing drug treatment in his opinion. An indwelling catheter was used for blood sampling during day 1, remaining blood samples were obtained by puncture of a forearm vein. The 5mL of blood were collected in evacuated polypropylene tubes containing sodium citrate solution 3.8% w/v. immediately after blood collection, the tubes were centrifuged for 10 min at 4000 rpm. After centrifugation, at least 2mL plasma were rapidly transferred into two polypropylene tubes and stored in appropriately labeled freeze resistant bags at −70◦C until sent to the analytical laboratory.

Pharmacokinetics and statistical analysis

All pharmacokinetic parameters of dextromethorphan were calculated using non-compartment methods: Cmax (maximum plasmatic concentration of DM) and Tmax (the time to reach Cmax) were obtained directly from the concentration– time curve. AUC0–t (area under the plasma concentration– time curve of dextromethorphan from zero to the last measurable sample time) was calculated using the linear trapezoidal method. Kel was calculated by applying a log–linear regression analysis to at least the last three concentrations of dextromethorphan. T1/2 (the terminal half-life) was calculated as 0.693/Kel (Ritschel, 1992).

RESULTS AND DISCUSSION

Specificity

The method exhibited excellent chromatographic specificity with no endogenous plasma interference at the retention time of dextromethorphan hydrobromide and IS. Representative chromatograms of human blank plasma and human plasma spiked with dextromethorphan hydrobromide (110 ng/mL) and IS, Low limit of quantization and Dextromethorphan hydrobromide in human plasma at 3 h after a single oral dosage of 60 mg are shown in Fig. 1, 2, 3 & 4. Dextromethorphan hydrobromide and IS were well resolved with good symmetry with respective retention times of 6.242 and 9.783 minutes and a total analytical time of less than 10 minutes.

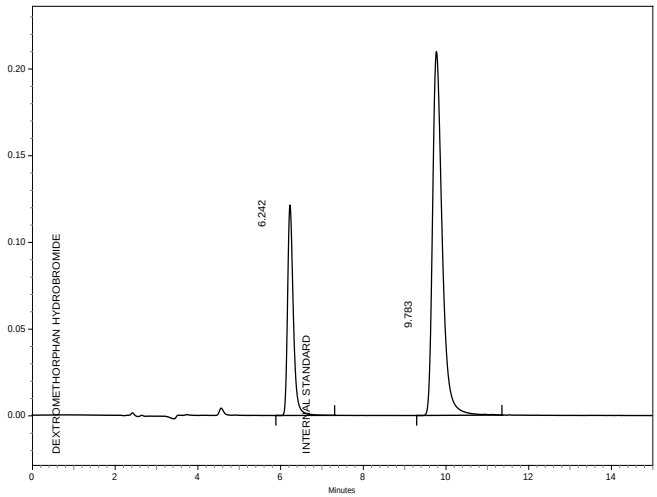

Fig. 1: Chromatogram of blank human plasma.

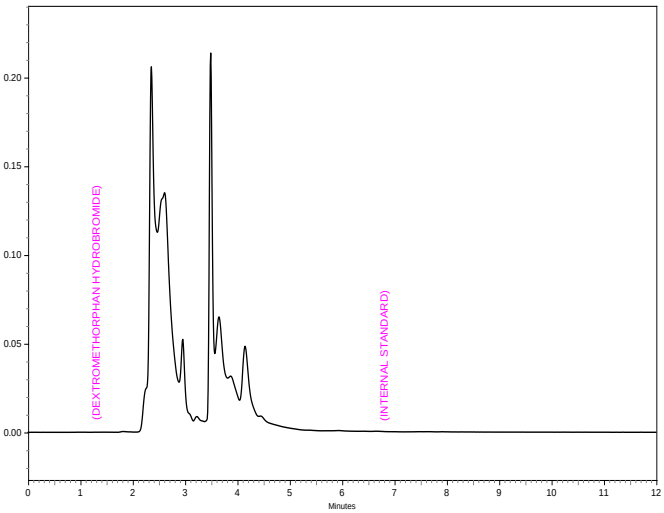

Fig. 2: Low limit of quantization chromatogram of Dextromethorphan hydrobromide in human plasma (110.0 ng/mL).

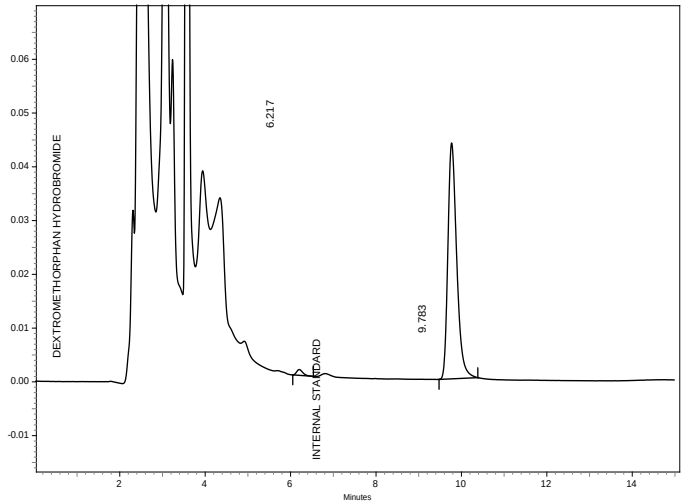

Fig. 3: Chromatogram of Dextromethorphan hydrobromide spiked in blank human plasma (0.0 µg/mL).

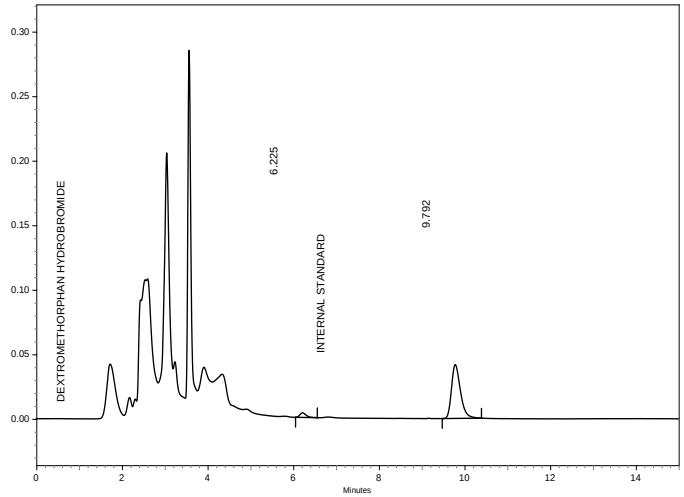

Fig. 4: Chromatogram of Dextromethorphan hydrobromide in human plasma at 3 h after a single oral dosage of 60 mg Dextromethorphan hydrobromide from a health volunteer.

Linearity and Range

Calibration standards containing 110-3000 ng/mL were prepared from working solutions of dextromethorphan hydrobromide and blank plasma. The calibration curve was constructed by plotting the peak area ratio of dextromethorphan hydrobromide to I.S. against the dextromethorphan hydrobromide concentration in plasma. A weighted linear regression method with a weight factor of 1/x was employed.

Precision, Accuracy and Recovery of the Method

Both the intra- and inter-day precision and accuracy of the method were determined by analysis of replicates (n = 5) of QC samples containing known concentrations of dextromethorphan hydrobromide. The precision of the method was described as relative standard deviation (RSD) among each assay. The accuracy of the method was evaluated by analysis of human plasma after administration of analyte spiked with standard solutions. The accuracy was described as a percentage error of measured concentrations versus nominal concentrations, recovery and the RSD, respectively. Precision and accuracy were calculated at each concentration. The results are shown in Tables No. 1. As can be seen from Table No. I, the method showed very good precision and accuracy. In this assay, the intra-day precisions (RSD) were within 3.38% and inter-day precisions were less than 3.72%. The intra-day accuracies (MRE) were within 5% and inter-day accuracies were less than 5%. This is satisfactory for the pharmacokinetic study.

Table No. 1: Accuracy Precision study of the HPLC method for determining concentrations of Dextromethorphan hydrobromide in human plasma.

Nominal concentration (ng\mL)	Mean found concentration Mean±S.D	Precision RSD (%)	Mean relative Error (%)
Intra-day			
110	110.25 ± 1.14	1.03	0.22
590	578.00 ± 19.55	3.38	-2.03
1400	1358.54 ± 27.72	2.04	-2.96
3000	2938.98 ± 45.89	1.56	-2.03
Inter-day			
110	108.17 ± 4.03	3.72	-1.66
590	581.43 ±8.87	1.52	-1.45
1400	1376.35 ±19.36	1.4	-1.68
3000	2892.61 ± 64.18	2.21	-3.57

Stability Study

Dextromethorphan hydrobromide was stable under conditions of storage and in the course of processing. Table No. II shows the results of stability. The QC plasma samples were stable for 24 h at room temperature (Table No. II). In the long-term stability study, the plasma samples spiked with the QC plasma samples also showed no loss of the analyte when they were stored for 30 days at ±20°C (Table No. 2). The stock solutions were stable for at least 1 month in the refrigerator. The final stability test was demonstrated after four freeze-thaw cycles. No significant deterioration of the analyte was observed under any of these conditions.

Table No. 2: Stability data for Dextromethorphan hydrobromide of quality control sample in human plasma (n=5)

Nominal concentration (ng\mL)	Mean found concentration Mean±S.D	Precision RSD (%)	Mean relative Error (%)
Short-term stability for 24 h in plasma at room temperature (STRT)			
590.00	585.43 ±7.98	1.36	-0.77
1400.00	1372.83 ±37.80	2.75	-1.94
3000.00	2899.38 ±127.97	4.41	-3.35
Storage in plasma at ±20°C for 1 month			
590.00	589.83 ±2.18	0.37	-0.02
1400.00	1412.23 ±32.19	2.28	0.87
3000.00	2839.38 ±80.73	2.84	-5.35
Four freeze/thaw cycles			
590.00	584.06 ±9.22	1.57	-1.00
1400.00	1375.48 ±43.83	3.18	-1.75
3000.00	2833.71 ±142.81	5.03	-5.54
Long Term Stability 40 days			
590.00	580.07 ±16.01	2.76	-1.68
1400.00	1344.55 ±21.25	1.58	-3.96
3000.00	2706.16 ±173.11	6.39	-9.79

Pharmacokinetic study

The pharmacokinetic study was performed in six humans after administration of dextromethorphan hydrobromide at a single oral dose of 60 mg. Plasma concentration-time curves of dextromethorphan hydrobromide after oral administration of DM are shown in Fig.5.

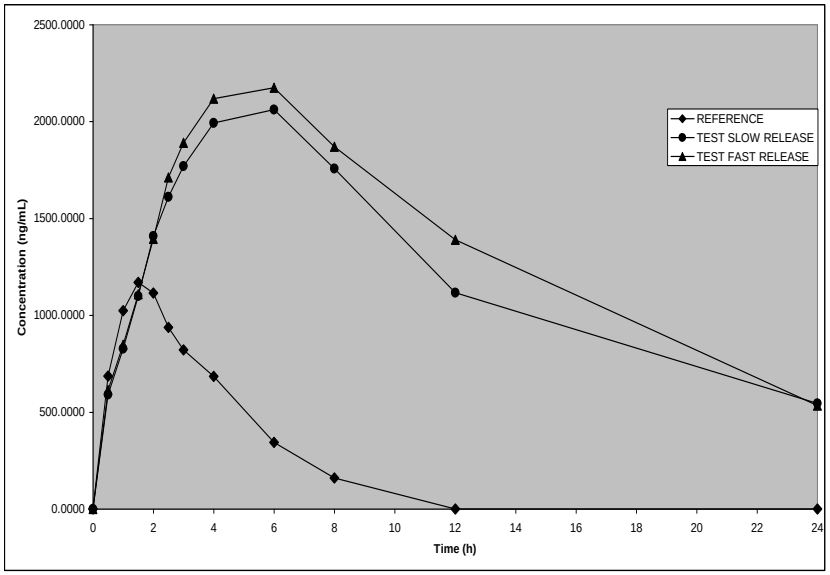

Fig. 5: Mean plasma concentration –time profile of Dextromethorphan hydrobromide in human plasma after oral administration of test and reference tablets (n=6).

The mean values of pharmacokinetic parameters and statistical moment parameters of SR and ST are summarized in Table 3. The Cmax of IR was significantly lower than that of SR and the Tmax was significantly longer, which suggested that the new SR possessed good sustained release properties.

Table 3: The mean pharmacokinetic parameters of Dextromethorphan hydrobromide in healthy human volunteers.

Parameters	Test		Reference
	Slow	Fast	
C_{max} (ng/mL)	2086.82±152.94	2191.67±108.58	1229.61±59.69
T_{max} (h)	6.33±0.82	5.33±1.03	1.67±0.26
$T_{1/2}$ (h)	9.62±0.73	9.16±0.86	2.52± 0.21
AUC_{0-t} (ng/mL)	28686.50±1828.40	31720±3681.88	5281.06±518.57
$AUC_{0-\infty}$ (ng/mL)	36289.19±2364.84	38845.90±3894.99	6537.59±781.23
K_{el}	0.07±0.01	0.08±0.01	0.28±0.02

CONCLUSION

The HPLC method presented here fulfils the general requirement for bioanalytical assays and is suitable for the pharmacokinetic study of dextromethorphan hydrobromide during preclinical drug development. The newly-developed SR of dextromethorphan hydrobromide with markedly lower peak plasma concentrations could improve its safety profile and allow for a more convenient dosing regimen.

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REFERENCES

1. Cranston JW and Yoast R. Abuse of Dextromethorphan. Archives of Family Medicine, 1999; 8: 99–100.
2. Roberge RJ, Hirani KH, Rowland PL, Brekeley R and Krenzelok EP. Dextromethorphan and Pseudoephedrine Induced Agitated Psychosis and Ataxia: Case Report. Journal of Emergency Medicine, 1999; 17: 285–288.

3. Silvati Y, Kartunen P, Tukiainen H, Kokkonen P, Hanninen U and Nykanen S.Pharmacok ineticsof Dextromethorphan and Dextrorphan a Single Dose Comparison of Three Prepar ation in human volunteers. International Journal of Clinical Pharmacology, Therapy & Toxicology, 1987; 25: 493-497.
4. El-Sayed YM, Niazy EM and Khidr SH. In-vivo Evaluation of Sustained-Release Microspheres of Metoclopramide Hydrochloride in Beagle Dogs. International Journal of Pharmaceutics, 995; 123: 113–118.
5. Hosny EA, Al-Helw ARM and Al-Dardiri MA. Comparative Study of In-vitro Release Diclofenac Sodium from Certain Hydrophilic Polymers and Commercial Tablets in Beagle Dogs. Pharmaceutical Acta Helvetiae, 1997; 73: 159–164.
6. Barnhart JW and Massad EN. Determination of Dextromethorphan in Serum by Gas Chromatography. Journal of Chromatography A, 1979; 163: 390–395.
7. Chen ZR, Somogyi AA and Bonchner F. Simultaneous Determination of Dextromethorphan and Three Metabolites in Plasma and Urine using High Performance Liquid Chromatography with Application to their Disposition in Man. Therapeutic Drug Monitoring, 1990; 12: 97–104.
8. Demirbas S, Reyderman L and Stavchansky S. Bioavailability of Dextromethorphan (as dextrorhan) from Sustained Release Formulati-ons in the Presence of Guaifenesin in Human Volunteers. Biopharmaceutics & Drug Disposition, 1998; 19: 541-545.
9. Kubiak EJ and Munson JW. Determination of Dextromethorphan Hydrobromide by High Performance Liquid Chromatography using Ion-Pair Formation. Journal of Pharmaceutical Sciences, 1980; 69: 1380-1384.
10. Menyharth A, Mahn FP and Heveran JE. Rapid Quantitative Determination of Three Active Ingredients in Liquid Antitussive Preparations by High-Speed Liquid Chromatography. Journal of Pharmaceutical Sciences, 1974; 63: 430-434.
11. McSharry WO & Savage IVE. Simultaneous High-Pressure Liquid Chromatographic Determination of Acetaminophen, Guaifenesi-n, & Dextromethorphan, Hydrobromide in Cough Syrup. Journal of Pharmaceutical Sciences, 1980; 69: 212-214.
12. Naomi Nagai, Takashi Kawakubo, Fumiyo Kaneko, Mika Ishii, Raito Shinohara, Yukihisa Saito, Hideo Shimamura, Akihiro Ohnishi and Hiroyasu Ogata. Pharmacokinetics and Polymorphic Oxidation of Dextromethorphan in a Japanese Population Biopharmaceutics & Drug Disposition, 1996; 17: 421-433.

13. Ramachander G, Williams ED and Emele JF. Determination of Dextrorphan in Plasma and Evaluation of Bioavailability of Dextromethorphan Hydrobromide in Humans. Journal of Pharmaceutical Sciences, 1977; 66: 1047–1048.
14. Ritschel WA. Handbook of Basic Pharmacokinetics Drug Intelligence Hamilton, 1992; 1–588.
15. Stavchansky S, Demirbas S, Reyderman L and Chai CK. Simultaneous Determination of Dextrorphan and Guaifenesin in Human Plasma by Liquid Chromatography with Fluorescence Detection. Journal of Pharmaceutical Biomedical Analysis, 1995; 13: 919–925.
16. William E. Smith, John D. Buehler and Manford J. Robinson. Preparation and In vitro Evaluation of a Sustained-Action Suspension of Dextromethorphan. Journal of Pharmaceutical Sciences, 1970; 59: 776-779.
17. Weber OWA, Heveran, JE. Automated. Simultaneous Determination of Dextromethorphan Hydrobromide, Glyceryl Guaiacolate, and Phenylpropanolamine Hydrochloride in cough syrups. Journal of Pharmaceutical Sciences, 1973; 62: 1174-1177.