Estimation of sulfolaxand antimicrobial preservatives in laxative drops

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Abstract: A simple, fast, precise, economic, selectiveand accurate HPLC method for simultaneous estimation of sorbicacid, sodium picosulphate and methyl parabensodium in laxative drops has been developed and subsequently validated. Chromatographic separation was achieved using gradient elution with mix phosphate buffer pH 7.0 and acetonitrile. The column used was purospherstar C18, 5µm, 25cm × 4.6mm kept at 25°C with 1ml/min flow rate using detection (PDA) at 263nm. The retention times of sorbicacid, sodium picosulphate and methyl paraben sodium were found to be 4.6, 7.4 and 11.4 minutes respectively. The proposed method was found to be linear over a concentration range of 8-12µg/ml for sorbic acid, 60-90µg/ml for sodium picosulphate and 16-24µg/ml formethyl paraben sodium respectively. The recovery was found to be 99.13-101.68% for sorbic acid, 99.81-100.21% for sodium picosulphate and 99.84-100.09% for methyl paraben sodium respectively. The limit of detection (LOD) for sorbicacid, sodium picosulphate and methyl parabensodium were found to be 0.032µg/ml, 0.337µg/ml and 0.131µg/ml respectivelyand limit of quantitation (LOQ) for sorbicacid, sodium picosulphate and methyl parabensodium were found to be 0.097µg/ml, 1.023µg/ml and 0.399µg/ml respectively. The method was validated with respect to specificity, precision, accuracy, linearity and robustness according to guidelines of ICH.

Keywords: HPLC (Hitachi PDA); sorbicacid; sodium picosulphate; methyl parabensodium.

INTRODUCTION

Pharmaceutical analytical chemistry is an important part in monitoring the quality of pharmaceutical products for safety and efficacy. The analytical chemistry span has enhanced to higher levels especially with the development in synthetic organic chemistry and other disciplines of chemistry including bioanalytical and biotechnology sciences. The increased emphasis in present use of analytical methods particularly involving advance analytical technology has not only made it possible to evaluate the contents of active pharmaceutical ingredients in dosage forms and API's but also to characterize, identify, explicate and quantify important constituents like active moiety, isomers, metabolites, chiral components, impurities and prophecy of the degradation products, i.e. impurities being produced. The appropriate test should be chosen for a particular purpose: sensitive methods are looked-for impurity profiling, selectivity is desirable for identity testing and good analytical repeatability is obligatory for the determination of content (also called assay) of drugs (Gorog *et al*., 2008).

The prologue and rapid increase of high-performance liquid chromatography is the most incredible development to facilitate the cause. It serves the dual purpose; at pharmacopoeial level its importance is in the quality compliance of bulk drugs and pharmaceutical preparations and increasingly important when coupled with mass spectroscopy for the determination of drugs along with their metabolitesin biological fluids (Gorog *et al*., 2007).

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Validation process is used to access the applicability of analytical methods. Validation is the official and methodical way to reveal the aptness of a developed method for testing the analyte to endow with useful analytical data within defined limits. Method validation studies encompass the overall course of action established during method development counting sample preparation, analysis and the estimation of the results. The applicability and the requirements mainly depend on the analyte being tested, the analytical method used and the region of the method (Ermer *et al*., 2001). To have obvious definitions of the different validation criteria used is therefore of key importance to judge this validity. To use statistical methods which are relevant with these definitions and accordingly to have methodologies in agreement with these definitions, the intent of validation and objective of analytical method are also indispensable (Rozet *et al*., 2007). A major inconsistency contribution in liquid chromatography originating from the standard preparation and analysis can be condensed after it has been fully established that the analytical system is stable under statistical control (Ermer *et al*., 2005).

Pharmacopoeias rely more on instrumental techniques rather than the classical wet chemistry methods. In the present research work a modest attempt has been made to develop validated analytical method for the determination of sorbic acid, sodium picosulphate and methyl paraben sodium in combined dosage form.

Sodium Picosulphate (SP) commonly known as sulfolaxis described chemically as 4,4'-((pyridin-2-yl) methylene) diphenylbis (sodium sulphate) with empirical formula $C_{18}H_{13}NNa_2O_8S_2$ (fig. 1). Molecular weight of sodium

picosulphate is 481.41. Sodium picosulphatebelongs to a medicine group known as laxatives. Sodium picosulphate is taken by oral route. The activity of sodium picosulphate is triggered by bacteria that are naturally present in the wall of large intestine. Nerve endings present in the wall of large intestine are stimulated to start a process known as peristalsis in which the nerves make the muscles of rectum and intestine contract more often and with more force. The content of rectum and intestine are moved along as a result of this increased muscle action resulting in bowl clearance and hence relieves constipation symptoms. To have an effect sodium picosulphate takes about 6-12 hours. To stimulate the emptying of the bowl before medical investigation of the gut, childbirth or surgery, sodium picosulphate is prescribed (Sweetman *et al*., 2005).

Sorbic Acid (SA) is described chemically as (2E, 4E)-hexa-2,4-dienoic acid with empirical formula $C_6H_8O_2$ (fig. 2). Its molecular weight is 112.14. To inhibit the growth of many bacteria, yeasts and most moulds, sorbic acid is an effective agent. To extend the shelf life of product by preventing microbiological growth, sorbic acid is widely employed in dairy, beverages and food products (Rowe, Sheskey and Owen, 2006).

Methyl Paraben Sodium (MPS) is chemically sodium methyl p-hydroxybenzoate with empirical formula $C_8H_7NaO_3$ (fig. 3). Molecular weight of methyl paraben sodium is 174.1. Being water soluble antiseptic, coupled with the features of high efficiency, security and broad-spectrum methyl paraben is widely used in food, textile and pharmaceutical industry; also used in the preservation of daily products, feed and cosmetics (Rowe, Sheskey and Owen, 2006).

Sorbic acid and methyl paraben sodium are official in United States Pharmacopeia and British Pharmacopoeia where as Sodium Picosulphate is official only in British Pharmacopoeia and both United States Pharmacopeia and British Pharmacopoeiadescribe titration methods for their individual estimation (USP, 2011; BP, 2012). British Pharmacopoeia describes an HPLC method for estimation of Sodium Picosulphate in oral solution (BP, 2012). The HPLC is still not the official method in any pharmacopeia for the analysis of sodium picosulphate and antimicrobial preservatives in the presence of degradation products.

It is apparent from literature survey that methods are quoted for determination of sorbic acid, sodium picosulphate andmethyl parabensodium individually by UV-VIS Spectrophotometry (Gend *et al*., 1973; Zonneveld *et al*., 1975), High Performance Liquid Chromatography (Wolff *et al*., 1981; Morton *et al*., 1987; Bui *et al*., 1987;Amati*et al*., 1997; Garcia *et al*., 2003; Wen *et al*., 2007; Santi *et al*., 2008;Savic*et al*., 2009),Gas Chromatography (Larsson *et al*., 1983; Coelho *et al*., 1983; Giryn *et al*., 1990), LC-MS (Negri *et al*., 2005; Yang *et al*., 2013), GC-MS (Thomas *et al*., 1999; Beyer *et al*., 2005), Capillary Zone Electrophoresis (Blanco *et al*., 2001; Zhang *et al*., 2011), Thin Layer Chromatography (Duncan *et al*., 1991) and High Performance Thin Layer Chromatography(Perkins *et al*., 1993) for single analyte but in combination not only a single method is available. To develop simple, rapid, accurate, specific and economic HPLC method for the simultaneous estimation of sorbicacid, sodium picosulphate and methyl paraben sodium in pharmaceutical dosage form (drops) is the objective of the present work.

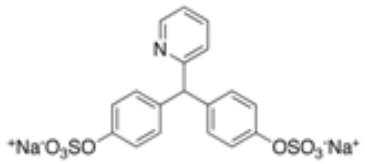

Fig. 1: Structure of sodium picosulphate

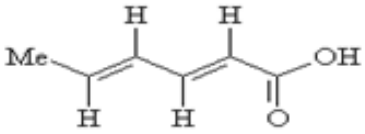

Fig. 2: Structure of sorbic acid

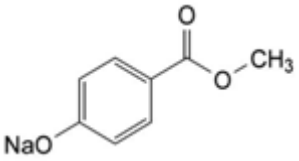

Fig. 3: Structure of methyl paraben sodium

MATERIALS AND METHODS

Instruments

High performance liquid chromatograph Hitachi L-2000 series equipped with quaternary gradient pump (L-2130), auto sampler injector (L-2200), diode array detector (L-2455) and column oven (L-2300) was used.

Materials and Chemicals

Standard bulk drug samples sorbic acid, sodium picosulphate and methyl parabensodium were provided by Merck KGaA Germany, Precise Chemipharma (Pvt.) Limited, India and Rasula Pharmaceuticals & Fine Chemicals, India respectively. Combined dosage form drops (Skilax Drops) were received from product development department of Highnoon Laboratories Limited,Lahore, Pakistan. HPLC grade reagents and solvents were used throughout study. Purospher star column C18, 5µm, 25cm × 4.6mm maintained at 25°C was used for HPLC method development. Eluent selected for this method contained 2.21gm disodium hydrogen phosphate dihydrate and 1.70gm potassium dihydrogen phosphate in 1000ml water; mix phosphate buffer of pH 7.0 (filtered through 0.45µm membrane filter) and

acetonitrile. 1ml/min flow rate was employed for elution. Detection of eluent was carried out at 263nm PDA detector. Standard stock solution of pure drugs was made in distilled water containing 10µg/ml of sorbic acid, 75µg/ml of sodium picosulphate and 20µg/ml of methyl parabensodium and filtered through a 0.22µm membrane filter. 20µl of standard solution was injected and a chromatogram was recorded. Mean retention times for sorbicacid, sodium picosulphate and methyl parabensodium were found to be 4.6, 7.4 and 11.4min respectively.

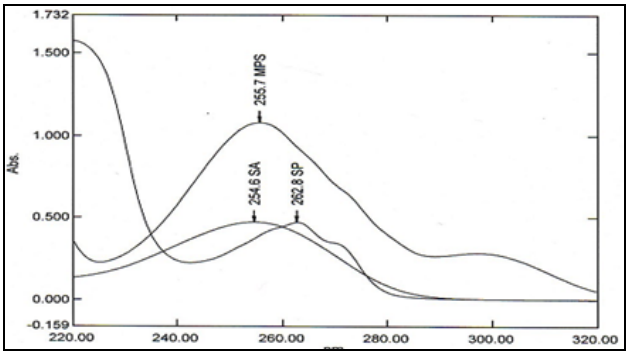

Fig. 4: Overlaid spectrum of sorbic acid, sodium picosulpfate and methyl paraben sodium

Analytical wavelength selection

Dilutions made in distilled water were scanned and absorbance maxima 263 nm was selected for analysis of sorbicacid, sodium picosulphate and methyl parabensodium. (fig. 4)

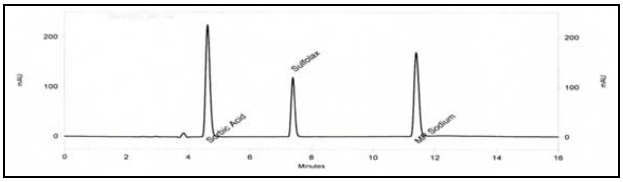

Fig. 5: Chromatogram of sample solution of sorbic acid (4.6min), sodium picosulphate (7.4min) and methyl paraben sodium (11.4min) at 263nm

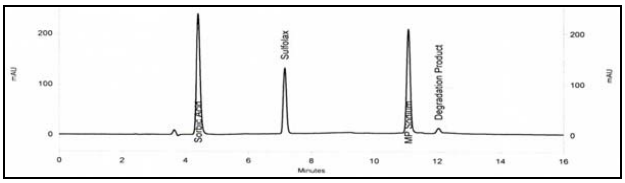

Fig. 6: Chromatogram of UV light degradation

Procedure for commercial formulation

Each ml of formulated drops was found to contain 1mg/ml sorbicacid, 7.5mg/ml sodium picosulphate and 2mg/ml methyl parabensodium. Two ml of drops were transferred to a 200ml volumetric flask and diluted with distilled water to volumeand filtered through a membrane filter (0.22µm). The proposed method was successfully applied to determine sorbicacid, sodium picosulphate and methyl parabensodium in their dosage form. The HPLC chromatogram for sorbicacid, sodium picosulphate and methyl parabensodium for sample is shown in (fig. 5). The results obtained for sorbicacid, sodium picosulphate and methyl parabensodium were comparable with the corresponding labeled amounts (table 1).

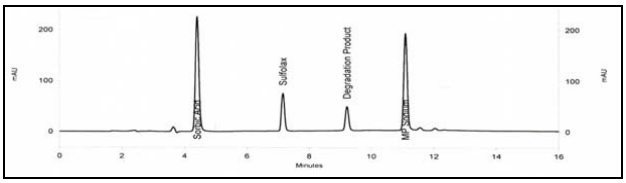

Fig. 7: Chromatogram of acid degradation

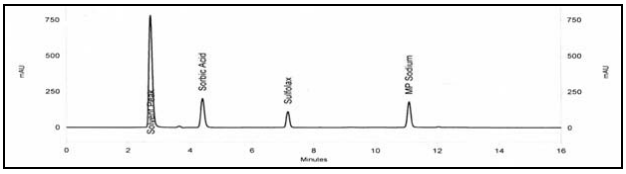

Fig. 8: Chromatogram of hydrogen peroxide degradation

RESULTS

Method development

Various compositions of mobile phase were tried to optimize the LC parameters. Among different solvents and mixtures of solvents investigated (methanol and acetonitrile in varying proportions with buffer at different conc. and pH values), gradient elution described in (table 2) furnished sharppeaks with excellent symmetry and desired resolution.

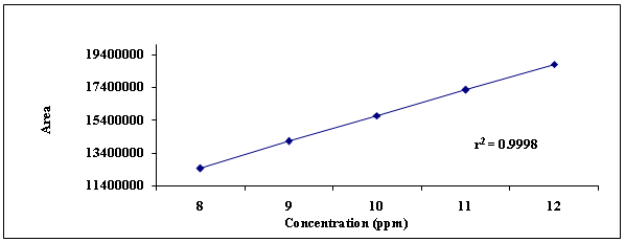

Fig. 9: Linearity graph of sorbic acid at 263nm

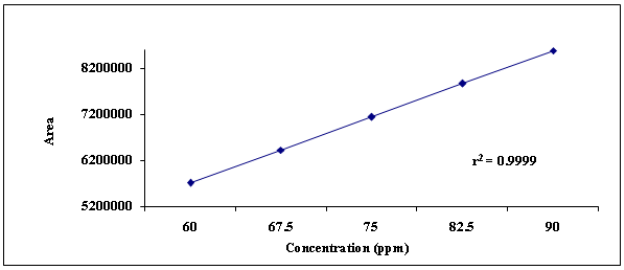

Fig. 10: Linearity graph of sodium picosulfate at 263nm

Several other mobile phases that were tried earlier did not give peaks in a short time with desired symmetry and required resolution was not painstaking. The concluding assortment on mobile phase opus and flow rate was ended

Table 1: Results of commercial formulation.

Sample	Labeled Amount (mg/ml)	Amount taken for assay (µg/ml)	Amount found* (µg/ml)	% label claim
Sorbic Acid	1	10	10.066 ± 0.153	100.66
Sodium Picosulphate	7.5	75	75.435 ± 1.191	100.58
Methyl Paraben Sodium	2	20	20.16 ± 0.304	100.80

on the foundation of baseline waft, peak outline (peak unevenness, peak area and asymmetry), time requisite for examination and price tag of solvent. Quantification was based on peak area and achieved with PDA detection at 263nm. The run time is just 15 minutes per sample. No interference of excipients present in the formulation was observed in the accurate estimation of sorbicacid, sodium picosulphate and methyl parabensodium.

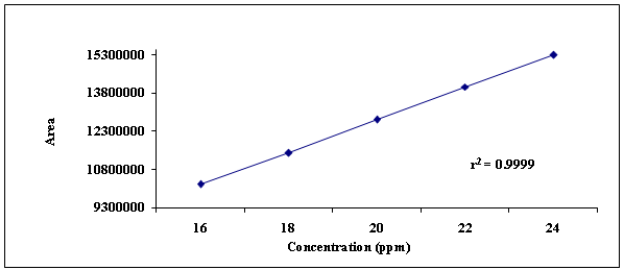

Fig. 11: Linearity graph of methyl paraben sodium at 263nm

Method validation

Method was validated according to ICH course of action with reference to specificity, precision, intermediate precision, accuracy, linearity, limit of detection, limit of quantitation, analytical solution stability and robustness. Results are revealed in (table 3).

Table 2: Gradient elution time programme.

Time (minutes)	Mix phosphate buffer pH 7.0 % v/v	Acetonitrile HPLC % v/v
0	85	15
9	50	50
14	85	15

Specificity

Specificity of the method was investigated by observing any intervention encountered from blank (diluent), placebo (formulation excipients) and impurity peaks with the main peak. The sample preparation was subjected to UV light, heat, hydrolysis, acid, base and oxidation. The purity index obtained is well within the limit of acceptance criteria (table 4).

Precision (Repeatability)

Precision was dogged at two levels, i.e. system precision and method precision. System precision determined by measurement of six replicates of bulk. Method precision determined by measurement of six replicates of sample. The percentage relative standard deviation was found to be 1.52%, 1.58% and 1.51% for sorbicacid, sodium picosulphate and methyl parabensodium respectively. The %RSD values specify that anticipated method is repeatable.

Intermediate precision (Reproducibility)

The course of action followed for method precision was repeated by a different analyst, on a different day, with a different HPLC system. Together intra-day and inter-day precision were dogged. There were no momentous differences between RSD (%) values for intra-day and inter-day precision, which indicates the method is reproducible.

Accuracy

The recovery experimentation was performed by the standard addition method. The accuracy of the method was established at three levels i.e. 80%, 100% and 120% of the established label concentration of the product tested and reported as the difference between the actual value found in the analyses and the theoretical value.

Linearity and range

The analytical concentration ranges over which the drugs obeyed Beer Lambert's law were found to be 8-12µg/ml for sorbicacid (r^2=0.9998), 60-90µg/ml for sodium picosulphate (r^2=0.9999) and 16-24µg/ml for methyl paraben sodium (r^2=0.9999). The standard calibration curves are given in (fig. 9, 10 and 11).

Robustness

The robustness of method was determined to appraise the upshot of miniature, but on purpose, disparity of the chromatographic setting on the determination of sorbicacid, sodium picosulphate and methyl parabensodium. Robustness was determined by varying; mobile phase flow rate, analytical wavelength, column temperature and column brand. The data indicates that there is no noteworthy divergence between the results obtained under customary condition and mottled method parameters. Therefore the method is robust.

Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection and limit of quantitation is derived from the residual standard deviation of regression line

Table 3: Validation results

Validation Parameters	Sorbic Acid	Sodium Picosulphate	Methyl Paraben Sodium
Specificity	Specific	Specific	Specific
Peak purity index	1.000	1.000	1.000
System Suitability			
Resolution factor (Rs)	---	14.30	21.50
No. of theoretical plates (N)	7256	24620	54823
Tailing factor (Asymmetry)	1.13	1.07	1.07
Precision (Repeatability)			
System* (%RSD)	0.98	1.09	1.10
Method* (%RSD)	100.66 ±1.52	100.58 ±1.58	100.80 ±1.51
Precision (Reproducibility)			
Intra-day* (% RSD)	100.66 ±1.52	100.58 ±1.58	100.80 ±1.51
Inter-day* (% RSD)	100.15 ±0.85	99.63 ±0.48	99.88 ±0.75
Accuracy			
Level I (n=3)(% RSD)	100.14 ±0.12	99.87 ±0.06	99.96 ±0.12
Level II (n=3)(% RSD)	99.76 ±0.07	99.99 ±0.08	99.94 ±0.05
Level III (n=3)(% RSD)	100.69 ±1.35	100.07 ±0.12	99.92 ±0.06
Linearity range (µg/ml)	8-12	60-90	16-24
r^2	0.9998	0.9999	0.9999
% Y-Intercept	-1.56	-0.24	0.09
Slope	6.2822×10^{-7}	1.0464×10^{-5}	1.5677×10^{-6}
Robustness (Cumulative %RSD)			
Change in flow rate			
0.8ml/min (n=3)	100.07 ±1.57	100.21 ±1.40	100.39 ±1.36
1.2ml/min (n=3)	100.44 ±1.38	100.32 ±1.31	100.28 ±1.45
Change in detection wavelength			
261nm (n=3)	100.39 ±1.28	100.21 ±1.37	100.24 ±1.44
265nm (n=3)	100.27 ±1.41	100.47 ±1.33	100.43 ±1.38
Change in column temperature			
23°C (n=3)	100.34 ±1.39	100.29 ±1.37	100.34 ±1.38
27°C (n=3)	100.18 ±1.45	100.10 ±1.50	100.24 ±1.50
Change of column			
Merck (n=3)	100.74 ±1.28	100.51 ±1.35	100.59 ±1.28
Teknokroma (n=3)	100.41 ±1.36	100.32 ±1.43	100.26 ±1.53
LOD (µg/ml)	0.032	0.337	0.131
LOQ (µg/ml)	0.097	1.023	0.399
Analytical Solution Stability (Cumulative % RSD)	1.44	1.49	1.48

based on detection limit co-efficient 3.3 and quantitation limit co-efficient 10 respectively. The data shows that the anticipated method is insightful for the detection and quantitation of sorbic acid, sodium picosulphate and methyl paraben sodium.

Analytical solution stability

The stability of the drug in solution during analysis was determined by recurring analysis of the sample solution prepared for precision study after storage of the drug solution for 24 hours under laboratory bench conditions against newly prepared reference solution to establish the solution stability period. The cumulative % RSD of content of initial analysis and solution stability were less than 2.0%, also no extraneous peak of impurities or degradants is observed in chromatogram of sample for chromatographic method proving the solution stability for the predetermined time interval.

DISCUSSION

The study was aimed at the simultaneous estimation of sorbic acid, sodium picosulphate and methyl paraben sodium by developing and validating a precise, reliable and economical, stability-indicating HPLC method, unlike the methods in literature for individual estimations of the said compounds (Wolff *et al*., 1981; Morton *et al*., 1987; Bui *et al*., 1987; Amati *et al*., 1997; Garcia *et al*., 2003; Wen *et al*., 2007; Santi *et al*., 2008; Savic *et al*., 2009). The data of assay method validation of laxative

Table 4: Interference from blank, placebo and impurities

Sample	Amount (µg/ml)						Peak Purity Index		
	Initial			Final					
	SA	SP	MPS	SA	SP	MPS	SA	SP	MPS
Blank (Diluent)	---	---	---	---	---	---	---	---	---
Placebo (Excipients)	---	---	---	---	---	---	---	---	---
Sample Unstressed	9.901	74.163	20.217	---	---	---	1.000	1.000	1.000
Sample Stressed									
Light	---	---	---	9.971	75.081	19.944	1.000	1.000	1.000
Heat	---	---	---	9.659	60.877	15.594	1.000	1.000	1.000
Hydrolysis	---	---	---	9.841	74.465	19.410	1.000	1.000	1.000
Acid	---	---	---	9.217	42.561	18.400	1.000	1.000	1.000
Base	---	---	---	9.336	69.972	10.756	1.000	1.000	1.000
Oxidation	---	---	---	8.420	64.291	17.291	1.000	1.000	1.000

drops for sorbic acid, sodium picosulphate and methyl paraben sodium by high performance liquid chromatography showed that the method is precise and reproducible.

The effect of UV light, acid and oxidative degradation on the specificity of the method was studied. In other studies, the thermal and oxidative stability, and alkaline hydrolysis of sodium picosulphate has been reported (Savic *et al.*, 2009; Savic *et al.*, 2010). The present study showed that there was no interference of diluent, mobile phase and placebo solution at the retention time of sorbicacid, sodium picosulphate and methyl parabensodium. The impurity peak was separated from the main peak. Peak purity for the main peaks and impurity peaks in stressed samples was not less than 0.99. Hence, the method isspecific. The method also fulfilled the requirements for accuracy, linearity and robustness (Ermer *et al*., 2005).

CONCLUSION

The parameters of system suitability are fitin acceptance criterion. Consequently system and chromatographic environment were apposite during each validation factor. Given that the outcomes are inside the approval criteria for every single validation parameter, for that reason method is measured as validated and appropriate for intended use. Also scheme is explicit for sorbicacid, sodium picosulphate and methyl paraben sodium in the attendance of degradation products (impurities) for that reason method is stability indicating. The standard deviation and standard error mean premeditated for the technique are squat, signifying towering measure of meticulousness of the method. In view of the fact that none of the methods is reported for instantaneous estimation of sorbicacid, sodium picosulphate and methyl parabensodium from combined dosage form, this developed method can be used for custom analysis of three components in formulation (drops).

ACKNOWLEDGEMENTS

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