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Simultaneous HPTLC Determination of Aceclofenac and Drotaverine Hydrochloride in Tablet Dosage Form

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Abstract: *A normal-phase simple, rapid and precise high performance thin – layer chromatographic method has been developed for simultaneous quantitative determination of Aceclofenac and Drotaverine hydrochloride in a tablet dosage form. The analysis was performed on Silica gel 60F_{254} on aluminum plates with acetonitrile – ethyl acetate – triethylamine, 2 : 7.6 : 0.4 v/v as a mobile phase. Detection and quantitation were performed densitometrically at wavelength 282nm. The developed method was validated for linearity, precision, solution stability, accuracy and robustness parameters. The linearity of Aceclofenac and Drotaverine hydrochloride were in the range of 50-150 µg/mL and 40-120µg/mL respectively. The correlation coefficient of Aceclofenac and Drotaverine hydrochloride were observed 0.9992 and 0.9995 respectively. Accuracy was checked by performing recovery studies from the pharmaceutical preparation. The average was found to be 99.48 ± 1.62% for Aceclofenac and 99.32 ± 1.52% for Drotaverine hydrochloride. The proposed HPTLC method was found to be accurate, precise and rapid for the simultaneous determination of Aceclofenac and Drotaverine hydrochloride in tablet dosage form.*

Keywords: Drotaverine hydrochloride, Aceclofeanc, HPTLC

I. INTRODUCTION

Aceclofenac which has molecular formula $C_{16}H_{13}Cl_2NO_4$, molecular weight 354.19. Aceclofenac (ACF), {[2-(2',6'-dichlorophenyl) amino] phenyl acetoxyacetic acid } is a new phenyl acetic acid [1] derivative with potent analgesic and anti-inflammatory properties with improved gastric tolerance. Drotaverine is an antispasmodic drug that works by inhibiting phosphodiesterase-4 (PDE4).5 It is a benzylisoquinoline derivative that is structurally related to papaverine, although it displays more potent antispasmodic activities than papaverine. Drotaverine Hydrochloride (DTV) chemically 1-[3, 4-(diethoxyphenyl)methylene]-6,7-Diethoxy-1,2,3,4-tetrahydroisoquinoline is an papaver analogue mainly used as antispasmodic and smooth muscle relaxant.[1]

Drotaverine has been used in the symptomatic treatment of various spastic conditions, such as gastrointestinal diseases, biliary dyskinesia, and vasomotor diseases associated with smooth muscle spasms. Literature survey revealed that various methods have been reported for the simultaneous determination of Aceclofenac and Drotaverine hydrochloride in pharmaceutical formulations,[1,3] viz, HPLC, UV and HPTLC [4-12]

II. EXPERIMENTAL

2.1 Working Standards and Chemicals

DTV and ACE working standard were obtained from ZESTICA Pharma (India). Tablet containing DTV(80mg) and ACE(100mg) were obtained from local market, AR grade methanol , acetonitrile and triethylamine were purchased from Merck India.

2.2 Instrumentation and Chromatographic Condition

The samples were spotted in the form of bands of width 5 mm with a desaga 100 µL sample syringe on silica gel precoated Al plate 60 F_{254}, with 200 µm thickness . These bands were applied with the help of Desaga AS 30- sample applicator at a distance of 10mm from X axis and 15mm from Y axis at the edge of the HPTLC plate with the speed of 150nl/sec for

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Quantitative assessment of Critic acid present in various soft drinks available in India.

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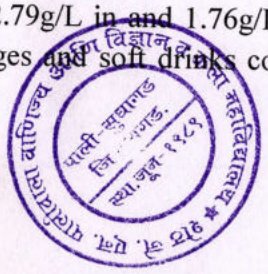
Abstract

Carbonated and non-carbonated are the two categories of soft/energy drinks are very commonly available. The non -carbonated soft drinks contain citric acid as a major component. In addition, some other acids such as Ascorbic, Malic, Caffeic acid are added in minute quantity as an as anti-oxidants, flavours and preservatives. In the current study the critic content is determined using two parallel methods for the same sample; one is visual titration and another by using pH-metre with combined glass electrode. The overall range for citric acid content is 1.445 to 9.722 g/L by visual titration whereas 1.577 to 1.577 by pH metric titration.

Introduction:

Soft drinks have five main subcategories viz bottled water; carbonated soft drinks; dilutables, (squashes, powders, cordials and syrups); fruit juices (100% fruit juice and nectars (25-99% juice content) and still drinks (including ready-to-drink (RTD) teas, sports drinks and other noncarbonated products with less than 25% fruit juice) [1]. Troiano et al. [2] reported that 20 - 24% of energy intake came from beverages. Some soft drinks are formulated to deliver a rapidly assimilated energy boost to the consumer. Soft drinks generally contain soluble sugars, which are easy to administer. To maintain the sour taste, to boost The Kerb cycle, to preserve the soft drink, to add a disinfectant in body, the multitasker component of utmost significance is 'citric acid'[3]. Phosphoric acid is added to cola drinks to enhance the tartness. A few lime tasting carbonated drinks also contain phosphoric acid for the same purpose [1]. Acidity regulators are the food additives used to change or maintain the pH whereas acidulants are the acids added to confer sour flavours [4]. The Citric acid plays double role as acid regulator as well as acidulant in the drink they contain. Acid like ascorbic acid is a Vitamin as well as an antioxidant. Malic acid, Fumaric acid and tartaric acid provide natural taste to the drink. Caffeic acid is another antioxidant used commonly [5]. Taylor [6] summarized that the components of soft drinks are as follows: water up to 98% v/v, carbon dioxide 0.30 - 60% m/v, acids 0.03 to 0.05% Sweetener (sugars 7-12 % m/v), colors (natural or synthetic) 0-70ppm. According to Md Nassiruddin et al,[7] the citic acid content in various types of leboos is 0.26 to 1.50 % and Vit C viz Ascorbic acid content is 1.36 to 3.39 %, Another study carried out by Peniston et al [8] showed that citric acid in lemon juice and lemon juice concentrate is 0.92 g/L and 48.0 g/L respectively. While its level in other commercially available juice products was ranging from 1.01 – 7.44g/L. Citrus fruits contain 0.026 to 1.50 % citric acid. Citric acid in energy drinks, juice drinks and soft drinks is found to be 7.3 g/L, 2.79g/L in and 1.76g/L respectively[3]. Generally, the concentration of citric acid in beverages and soft drinks commonly found is



same as in in natural fruits. Citric acid content of the fruits is as follows: orange 1%, grapefruits 1.5%, and lemon 2.5%. A typical addition of 0.25 to 0.4 % of citric acid is done to enhance flavour and as preservative as declared by European Citric Acid Manufacturers Association (ECAMA) [9]. There are no recommended concentration levels of citric acid in soft drinks; however USA soft and fruit drinks contain varying quantities of the same are 0.131- 0.350% and 0.6 – 6% respectively[10].

Materials and Methods:

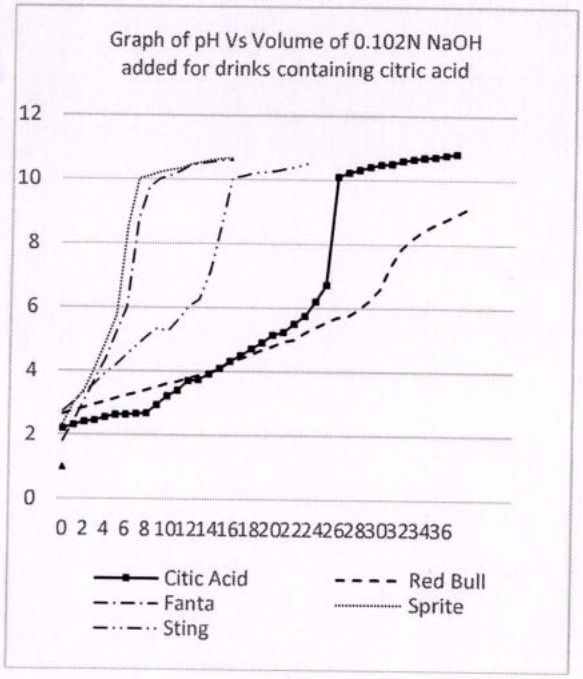
1. Various soft drinks bottles were purchased from market. To prepare the analysis sample, the bottled drink is poured in a 250 cm^3 borosil beaker and boiled for ten minutes to remove carbon dioxide or nitrogen if used during packing.
2. All the reagents used are of AR grade. Sodium Hydroxide solution is standardised using 0.1N K-H Phthalate solution. An aliquot of 25 cm^3 of sample is titrated with standard NaOH solution in both methods. Visual titration is done by using Phenolphthalein indicator solution. Similarly, Standard determination is done for Citric Acid.
3. The pH-meter with combined glass electrode is standardised by using the buffer solutions of pH= 7, pH = 9.2 and pH = 4. The titration of samples and standards was continued till pH of the titter solution exceeds the pH value of 10 and remains constant for further additions of titrant.
4. Two types of graphs are plotted in instrumental method. pH Vs Volume of NaOH solution added and $\frac{\Delta pH}{\Delta V}$ with volume of NaOH solution added. The first derivative graph is used to determine the equivalence point whereas the titration curve confirm nature of acid in the soft drink

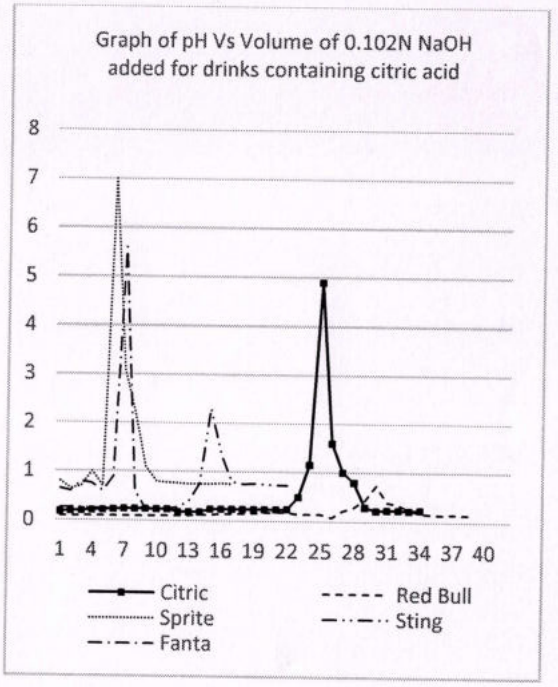
Results:

The estimation is done by two different ways to eliminate determinate error:
Running parallel determination and Comparison with standard.
The pKa values for citric acid are: $pKa_1 = 3.15$, $pKa_2 = 4.77$, $pKa_3 = 6.40$[11]. Visual titration gives a single end point corresponding to the third dissociations of citric acid. The indicator phenolphthalein shows colour change between pH range of 8.2 to 10. During titration with NaOH, this range corresponds to third dissociation. One drop in excess is sufficient to bring colour change at equivalence point of titration. Hence, equivalent weight is one third of the molecular weight of citric acid. Pure citric acid shows only one peak corresponding to third dissociation. Samples containing citric acids result into the graph with similar nature.
Since the other acidic ingredients are neutralised simultaneously, the end point corresponds to the total acid contents instead of specifically citric acid. Molecular weight of citric acid is 192.124 and that of Malic, Ascorbic and caffeic acid are in the same range [14] hence the total acid content is calculated in terms of citric acid as it is done for total hardness of water [15]. Using these stoichiometric relations, the acid contents of soft drinks is calculated.

Sr. No.	Name of drink	Strength of critic acid by Visual titration	Strength of citric acid by pH metric titration
1	Maza	3.100	3.153
2	Real Mango	3.757	3.731
3	Fruity	3.495	3.153
4	Slice	2.628	2.522
5	Appy	2.890	2.733
6	Mirinda	2.759	2.417
7	Sting	4.020	3.757
8	Limca	1.655	1.682
9	Sprite	1.445	1.577
10	Nimbooz	5.386	5.413
11	Fanta	2.102	1.839
12	7 up	1.787	1.629
13	Dawat Jeera	1.655	1.419
14	Rio (Mango)	2.838	2.733
15	Rio (Wild Berry)	2.890	2.733
16	Lichi	2.628	1.892
17	Red Bull	9.722	8.671
18	Bubbly Bhai	3.284	3.258
19	Dawat Lemon	3.284	3.258
20	Mint Monjito	2.759	2.785
21	Blaster Jeera	3.416	3.311
22	Alofruit Blood orange	3.810	3.836
23	Alofruit Kivi	2.759	3.100
24	Alo fruit Mosambi	4.730	4.835
25	Alo fruit Guava	2.890	2.943
26	Pure Citric acid	2.522	2.522

The overall range for total acid content is 1.445 to 9.722 g/L by visual titration whereas 1.577 to 8.671 by pH metric titration.





Discussions:

The acids are usually associated either their salts which sets a buffer system in the sample. Secondly, the flavours, antioxidants, Vitamins etc are either acids or salt which contribute to pH of sample. The titration when carried out using pH meter encounters these compounds may result in end point little different than that of volumetric. However, this difference is very small since the concentration of those ingredients is very low. The pH-metry helps to confirm the presence of citric or phosphoric acid from the graph.

This estimation is more valuable since the acid regulators and acidulants are neither mentioned by their names nor is their concentration printed. A common science student would not know the acid content of the drink whereas one can read the composition of a pharmaceutical. There is a numbering system used in Europe for all approved additives which is now adopted and extended internationally by Codex Alimentarius [12]. For example, Phosphoric acid as E330, Acetic acid as E 260 etc. In some countries other than Europe, the letter E is replaced by INS (International numbering system) or somewhere, E is dropped and simply number is used. The observation table contains the number same as mentioned by manufacturer. The United states Food and Drug administration (FDA) lists these items as 'Generally Recognized As Safe'[13]

Conclusions:

Using two different methods, classical as well as instrumental, the acid content estimated in ready to consume soft drink is between 3.613 ± 0.15 to 24.305 ± 0.27 for citric acid and for phosphoric acid containing drinks it is 2.499 ± 0.07 to 9.495 ± 0.35.There is no upper limit for concentration of citric acid in soft drinks in any guideline in any country. This concentration range is coinciding with that of natural fruit juices rather towards lower range. Since the fruits and fruit juices are perishable, the availability of synthetic drinks is winning the game against

the natural drinks. The ever-growing popularity of soft drinks is not just for the sake of taste or due to effect of advertisement but also its benefit for health. Quick thirst for refreshing nutrients like citric acid, vitamin C, sugar etc is satisfied by these drinks. However, since they do not contain enzymes, fibre and many micronutrients as available in natural drinks, the soft drinks cannot replace the natural juices.

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IV. Results and Discussions

4.1 Optimization of the Chromatographic Conditions

In order to develop a normal phase HPTLC method for the determination of Aceclofenac and Drotaverine hydrochloride in combined dosage form the chromatographic conditions were optimized. For better separation and resolution, the mixtures of different solvents of varying polarity were tried.

The different compositions of mobile phases were changed for getting better separation of analytes. Initially, chloroform –ethyl acetate 4 : 6 (v/v) and acetonitrile, toluene 5 : 5 (v/v) were used. The best results were obtained by the use of acetonitrile, ethyl acetate and triethylamine in the ratio of (2 : 7.6 : 0.4v/v/v). This mobile phase showed good resolution and separation of ACE and DTV peak from other formulation components or excipients tested as seen in fig 2.

Densitometric scanning of all the tracks showed compound with Rf value 0.38 ± 0.05 for Aceclofenac and 0.59 ± 0.04 for Drotavereine HCL. As the separation was takes place in a short time period the proposed method is quicker as compare to reported method.

Parameters	Chromatographic conditions
Development chamber	Twin trough chamber
Stationary phase	Silica gel
Mobile Phase	Acetonitrile : Ethyl acetate : Triethylamine (2 : 7.6 : 0.4v/v/v)
Chamber saturation	15 min
Sample applicator	AS 30 - SAMPLE APPLICATOR
Band	8mm
Space	12mm
Scanning speed	20mm/sec
Development distance	8 cm
Drying of plate	Room temperature
Densitometric scanner	CD 60 - DENSITOMETER / SCANNER
Lamp	Deuterium
Wavelength	282 nm
Volume	10µl

Table 1: Optimized chromatographic conditions

4.2 Method Validation

A. Linearity and Range

Linearity was observed over the concentration range of 50 - 150µg/mL for ACE and 37.5 – 112.5µg/mL for DTV (Table 2). The linearity was confirmed by the high value of the correlation coefficients of r^2= 0.9992 for ACE and 0.9995 for DTV

Table 2. Linear regression data

Drug	Linearity range	Correlation coefficient (r^2)	Slope	Intercept
Aceclofenac	50 - 150µg/mL	0.9992	3.250	-6.485
Drotaverine HCl	40 - 120 µg/mL	0.9995	7.846	-12.75

B. Precision

The developed method was validated for system precision and method precision. The precision study of the proposed method gave the results in the prescribed limits of relative standard deviation. This is less than 2 % for both analytes. The low value of RSD showed that the proposed method was reliable and reproducible.

Table 3: Precision study for Paracetamol and Mefenamic acid

Obs No	Aceclofenac		Drotaverine HCl	
	Peak Area	% Assay	Peak Area	% Assay
1	2450	101.61	1051	101.50
2	2475	101.50	1075	99.82

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3	2445	101.05	1085	98.92
4	2499	100.80	1045	99.34
5	2442	98.98	1048	97.94
6	2482	99.78	1105	99.80
	Mean	100.62	Mean	99.55
	S.D	1.036	S.D	1.180
	%R.S.D	1.029	%R.S.D	1.186

C. Solution Stability

Stability of a sample solution was checked by using sample prepared in the precision study. The sample solution was stored at room temperature for 24 hrs then it was withdrawn at the intervals of 2 hr, 4 hrs , 12 hrs and then applied on the chromatographic plate stored at room temperature for 24 hours, withdrawn at the intervals of 2hrs, 4 hrs, 12 hrs and 24 hrs and then applied on the chromatoplate.

After development, the chromatogram was evaluated for additional spots if any. There was no indication of compound instability in the sample solution. The results shows that the solutions were stable for 24 hrs at room temperature.

D. Specificity

An investigation specificity was conducted during the validation of identification tests, the determination of impurities and the assay. Demonstration of specificity requires that there should not be any interference of impurities and excipients. In practice this was done by taking the chromatogram of sample solution and the assay result was unaffected by the extraneous material. It has been found that there was no interference of the diluents, placebo at the Rf value of the analytes.

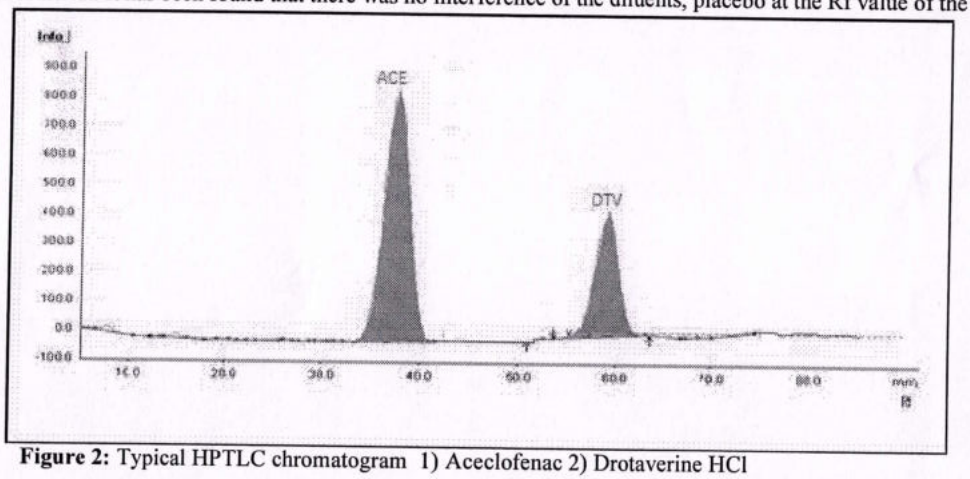

Figure 2: Typical HPTLC chromatogram 1) Aceclofenac 2) Drotaverine HCl

E. System Suitability Test

A system suitability test should be carried out to see if the HPTLC system is performing properly. System suitability tests were carried out as per the USP to confirm the suitability and the reproducibility of the system. The experiment was carried out using 100% level mixed standard solution of ACE and DTV. This solution was spotted five times on the chromatographic plate under the optimized chromatographic conditions. % RSD of the peak area shows that (Table 4) the proposed method was suitable for the system.

Table 4: System suitability for Aceclofenac and Drotaverine HCl

Obs No	100% Level			
	Aceclofenac		Drotaverine Hydrochloride	
	Peak area	Rf value	Peak area	Rf value
1	2433	0.38	1051	0.59

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2	2450	0.39	1102	0.60
3	2451	0.39	1086	0.59
4	2459	0.39	1075	0.61
5	2425	0.38	1015	0.60
Mean	2443	0.386	1065	0.598
S.D	14.06	0.0054	33.92	0.0083
% RSD	0.5755	1.418	3.18	1.399

4.3 Accuracy (Recovery Experiment)

The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100% level was considered as a zero level and 10% , 20% and 30% of the standard drug of ACE and DTV were added respectively. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for ACE and DTV from the sample solution are shown in Table 5 and 6. The results are within the acceptance limit and hence the method is accurate.

Table 5: % Recovery of ACE

Amount of Aceclofenac in ppm								
Sr. No	% Added	Original amount	Added amount	Total amount	Mean (n = 5)	% Recovery	S.D	% RSD
1	10	100	10.40	110.40	109.98	99.61	0.3881	0.3886
2	20	100	20.25	120.25	120.05	99.83	0.4922	0.4996
3	30	100	30.10	130.10	130.78	100.52	0.645	0.651

Table 6: % Recovery of ACF

Amount of Aceclofenac in ppm								
Sr. No	% Added	Original amount	Added amount	Total amount	Mean (n = 5)	% Recovery	S.D	% RSD
1	10	80	10.35	90.35	90.41	100.06	0.042	0.044
2	20	80	20.12	100.12	99.94	99.82	0.127	0.128
3	30	80	30.16	110.16	109.92	99.78	0.169	0.172

V. Conclusion

The HPTLC method for the determination of Aceclofenac and Drotaverine HCL from their tablet dosage form was found to be accurate, precise, specific and rapid. The results of the recovery studies show the high degree of accuracy of the proposed method. The advantage of the proposed method is that it require less time and cost effective method. Solvent consumption during the analysis is less. Therefore the proposed method can be applied successfully in routine analysis.

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