SIMULTANEOUS DETERMINATION OF PARACETAMOL WITH DIFFERENT ACTIVE PHARMACEUTICAL INGREDIENT (API) AND EXCIPIENT IN VARIOUS DOSAGE FORMS

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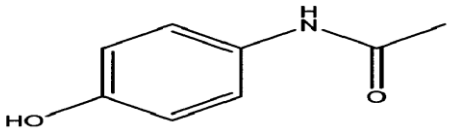
ABSTRACT: *The present study indented towards the simultaneous HPLC determination of paracetamol with a various range of API's (Active Pharmaceutical Ingredient) like caffeine, Phenobarbital, acetylsalicylic, Phenobarbital, acetylsalicylic, Triprolidine HCl, Peudoephedrine HCl, Dextromethorphan, ibuprofen, Camylofin dihydrochloride, phenylephrine hydrochloride, diphenhydramine hydrochloride, salicylamide, guaifenesin HCl, chlorpheniramine maleate, and promethazine hydrochloride and Excipient like sodium benzoate, propyl paraben, Para-aminophenol, 4-chloroacetanilide and Methyl paraben in different dosage form. Six methods are reviewed and discussed for the simultaneous determination of paracetamol . First method was related to HPLC chromatoraphic assay of caffeine, acetylsalicylic acid, paracetamol, Phenobarbital in tablet formulation is performed using RP-HPLC technique with column C18 ,mobile phase acetonitrile and water with adjusted pH 2.5 having flow rate 2.0 ml/min at 207nm. Second method was the assay of paracetamol and acelofenac, a RP-HPLC method at 265 nm using Hichrome C18, mobile phase acetonitrile and phosphate buffer having 0.8 ml/min. Third method was the estimation of triprolidine, pseudoephedrine, Dextromethorphan and Paracetamol where chromatography is carried put through C18 RP-HPLC Technique first at 254 then 280 nm. Fourth method was for the ibuprofen and paracetamol having system parameter comprises of C18 column with eluent pH 7 (acetonitrile and phosphoric buffer), and elution at 0.8 ml per min .Peaks was detected through UV detector 260nm.Fifth method was for paracetamol and camylofin dihydrochloride with water C18 column at 220 nm using methyl paraben as internal standard. Last method but no least was done through gradient system at 215nm with 40 min run time. These six assay procedures can be used in any pharmaceutical lab for the analysis of paracetamol combination in tablet, syrup, suspension and bulk.*

Keywords: Paracetamol, Simultaneous assay, HPLC, API's, Preservative

1. INTRODUCTION

Paracetamol that is also known with other name acetaminophen [1, 2] is an OTC medicine that is used to treat fever, minor aches and pain in adults as well as in children. It is an active ingredient in most of the medication given in cold. Severe pains are treated by combination of Paracetamol opioids such as cancer pain and use to treat after surgical



Paracetamol

discomforts [3]. It is not an NSAID due to its weak anti inflammatory activity it is classified as mild and analgesic and comes under the category of OTC because it is save to use in its recommended doses, While the over doses can be fetal as compared to other OTC medicine [4]. It has an empirical formula C8H9NO2 having IUPAC name N-(4-hydroxyphenyl)-ethanamide.

Paracetamol in combination with other Active ingredient as well as excipient, manufacture in different dosage form. One of the important excipient in oral liquid dosage form is preservatives. Different preservative is utilize for this purpose like, methyl paraben, sodium benzoate, Ethyl paraben, propyl paraben. Preservative make the medicine drug stable [5, 6].

Cough and cold is being treated by paracetamol and caffeine in tablet formulation [7]. In liquid it is use to treat cough and cold in addition with Triprolidine HCl, Peudoephedrine HCl and Dextromethorphan [8]. Quantitative determination of paracetamol drugs is carried out through HPLC, UV, titrimetry,Volumetry, GC and NIR [9, 10, 11, 12]. The present study is directed toward the review of simultaneous HPLC analysis of paracetamol combination with caffeine, Phenobarbital, acelofenac, triprolidine, seudoephedrine, acetyl salicylic acid, dextromethorphan and ibuprofen in different pharmaceutical dosage form.

2. MATERIAL AND METHODS

A simultaneous HPLC assay of caffeine, paracetamol, phenobarbital, and acetylsalicylic acid in tablet formulation is performed by *franeta et al*, where chromatographic system comprises of BioRed 18 01 pump, 71 25 rheodine injector and UV visible detector of BioRed is used for the separation. The column use was C18; 5 micron with mobile phase comprises of acetonitrile and water in a ratio of 25:75 V/V. Adjust pH around 2.5 which is adjusted with phosphoric acid. The chromatographic was run with flow rate 2.0 ml per minute on wave length 207 nm. A successful separation is achieved through this set of chromatography [13].

Another study of simultaneous HPLC assay of paracetamol and acelofenac was performed by *Gopinath et al.*, in tablet. It was an RP -HPLC method where the chromatography was carried out by Hichrome C18 (25 x 4.6) micron meter column and the eluent was a mixture of acetonitrile and 20mM phosphate buffer with the combination of 60: 40 V/V. 0.8 ml per minute with UV detection of 265 nm. The assay performed by using and internal standard Etoricoxib and it was noted that the retention time of paracetamol, acelofenac and Etoricoxib were 4.75, 6.44 and 8.8 minutes respectively [14, 15]. This was the first successful method for the evaluation of both of the molecules in tablet formulation [16, 17].

Orsi et al. has performed HPLC evaluation of another combination of API in diferent dosage form, developed the method of simultaneous determination of triprolidine, pseudoephedrine, Dextromethorphan and Paracetamol where C18 RP-HPLC Technique was used with a UV detection of 254 and 280 nm with a gradient evaluation, a successful separation resulted with a simple preliminary extraction procedure for tablet and cream and no extraction for liquid formulation [18, 19].

A simple & efficient HPLC method for the simultaneous assay of ibuprofen and paracetamol was developed and validated by *battu and reddy* [20, 21, 22]. It was carried out by using isocratic system, C18 column (150 mm x 4.6 mm) 5 micron with mobile phase acetonitrile and phosphoric buffer (60:40) v/v having pH 7.0 and elution rate 0.8 ml/min. Peaks were detected through using UV detector 260nm.consistancy and system suitability was sustained through using internal standard. Retention time of ibuprofen, paracetamol and acelofenac is found to be 2.48, 4.45 and 6.34 minutes respectively.

Singh et al. have worked on the separation of paracetamol and camylofin dihydrochloride in oral dosage form by using methyl paraben as internal standard. Sample preparation was carried out by serial dilution method. USP standards were used for paracetamol and camylofin dihydrochloride. Paracetamol and camylofin dihydrochloride was separated by passing sample injection through water C18 column, 50 % v/v organic (acetonitrile) solution of 0.05 % tirfluoroethanoic acid is used for the elution of sample through column at rate of 1 ml/min detected at 220 nm. Peaks were detected through photo diode array detector Paracetamol, Camylofin dihydrochloride and Methyl paraben were eluted at 3.42, 3.10 and 4.70 minutes respectively. System suitability was successfully met with RSD LT 2 % [23, 24, 25, 26, 27].

Shabrawy *et al*. have developed an HPLC method for simultaneous determination of paracetamol with 14 components (phenylephrine hydrochloride, pseudoephedrine hydrochloride, salicylamide, guaifenesin HCl, sodium benzoate, methyl paraben, chlorpheniramine maleate, triprolidine hydrochloride, dextromethorphan hydrobromide, diphenhydramine hydrochloride, promethazine hydrochloride, propyl paraben, Para-aminophenol and 4-chloroacetanilide) in anti-cold pharmaceutical products [28, 29, 30, 31, 32]. All components peaks have been separated well and eluted within a 40 min run time by using C18 symmetry column with gradient system at 215 nm. First M. phase was acetonitrile and other one comprised of buffer sodium dihydrogen phosphate, hexane sulfonic acid with pH 3.0 in tune with ortho-phosphoric acid. Ion pair reagent hexane sulfonic acid was used to amplify retention time and for better separation. Acetonitrile is used as diluents for preparation of standard [33, 34, 35].

3. RESULTS AND DISCUSSION

HPLC Study of paracetamol combination (caffeine, Phenobarbital, and acetylsalicylic acid) by *Franeta et al*, are presented in Table 1 [13].

Table 1: Simultaneous HPLC assay of caffeine, paracetamol, Phenobarbital, and acetylsalicylic acid in tablet formulation

Parameter	Results
Linearity	R > 0.998
Intraday precision	RSD=0.36–1.89 %
Inter day precision	RSD=0.58–2.18 %
Sensitivity	LOD: 9×10^{-5}–1.7×10^{-4} mg ml LOQ: 2.5×10^{-4}–5.6×10^{-4} mg/ ml
Accuracy	98.35–99.14 %
Reproducibility	Acetylsalicylic acid: 98.74–102.08 % Paracetamol 99.93–102.11 % Caffeine 98.25–102.12 % Phenobarbital 98.15–102.3 % RSD: 1.21–1.85 %

RP-HPLC determination of triprolidine, pseudoephedrine, Dextromethorphan and Paracetamol by *Orsi et al.* was validated with recovery 96 % to 98.7 % [18].

Gopinath et al. validation results of paracetamol and acelofenac are presented in Table 2 [15].

Table 2: Simultaneous HPLC assay of paracetamol and acelofenac

Parameter	Results
Linearity	Paracetamol : 20 µg/ml to 80 µg/ml Acelofenac : 0.5 µg/ml to 3.5 µg/ml
Slope and Intercept	Paracetamol Equation y =0.0072x-0.001; R^2 =0.998 Acelofenac Equation : y=0.0252x +0.003; R^2 =0.996
LOD	Paracetamol : 5 ng/ml Acelofenac : 10 ng/ml
signal to noise	10
LOQ	Paracetamol 15 ng/ml Acelofenac 30 ng/ml
system suitability parameters	within ±3% standard deviation

Validation and system suitability studies of tablet contain paracetamol and ibuprofen by *Reddy and Battu* are presented in Table 3 [20].

Table 3: Simultaneous HPLC assay of paracetamol and ibuprofen

Parameters	Paracetamol	Ibuprofen
Linearity	20 to 80 µg/ml	10 to 70 µg/ml
Equation of Regression	y = 0.0071x-0.001	y = 0.0061x+0.002
Correlation coefficient	0.999	0.998
Theoretical plate/meter	26458	28764
Resolution	1.30	1.30
Asymmetric	0.90	1.01
Tailing factor	1.2	1.0
LOD	6 ng/ml	10 ng/ml
LOQ	15 ng/ml	25 ng/ml

System suitability criteria for *Singh et al. method* was met with resolution 1.88 between paracetamol and Camylofin dihydrochloride (Table 4) and 6.55 for paracetamol and methyl paraben. Tailing factor for all peaks were less than 1.35. Theoretical plates were found to be 4242, 4745, 7760 for Camylofin dihydrochloride, paracetamol and methyl paraben respectively [27].

Table 4: Simultaneous HPLC assay of paracetamol and Camylofin Dihydrochloride

Parameters	Paracetamol	Camylofin Dihydrochloride
Linearity	R^2=0.9992	R^2=0.9993
Precision	RSD % 0.80	RSD % 0.80
Slope	0.011	0.004
Intercept	0.102	0.066
LOD	0.3 mg/ ml	0.25 mg/ ml

Validation of Shabrawy *et al.* method was performed with the parameter linearity, Precision and accuracy, range, detection, quantitation limit, specificity and range. Correlation coefficient was 0.9999 for all 15 components (Table 5). Quantitation limit in µg/ml for all 15 components were range of 0.01 - 0.3. Slope and Intercept of paracetamol with this method was found to be 2.26 x 10^4 and 1.19 x 10^2 statistical results are as follow [28].

Table 5: Simultaneous HPLC assay of paracetamol with 15 components

Parameters	Paracetamol
Range of calibration	0.1 to 150 µg per ml
Detection of limit	0.01 µg per ml
Quantitation limit	0.03 µg per ml
S.D of intercept	1.39 x 10^2
S.D of Slope	5.4

4. CONCLUSION

Paracetamol is an OTC medicine; it along with various API's and Excipient is quantified through different assay techniques. The present study is directed toward a simultaneous HPLC assay of paracetamol in different dosage form with different API's (caffeine, Phenobarbital, acetylsalicylic, Phenobarbital, acetylsalicylic, Triprolidine HCl, Peudoephedrine HCl, Dextromethorphan, ibuprofen, Camylofin dihydrochloride, phenylephrine hydrochloride, diphenhydramine hydrochloride, salicylamide, guaifenesin HCl, chlorpheniramine maleate, promethazine hydrochloride) as well as with Excipient (sodium benzoate, propyl paraben, Para-aminophenol, 4-chloroacetanilide and Methyl paraben). The above discussed methods are validated and is found simple, robust, accurate, reliable, reproducible and can be easily adopted in routine analysis. Pharmaceutical laboratories can easily adopting above methods for the chromatographic HPLC evaluation of paracetamol with above mentioned combinations. In present study review of different research papers has been performed for the assay of paractamol in combination of different API's and Excipient through HPLC, as per best of our knowledge there is a room of further work on the simultaneous assay development of paracetamol with preservative through HPLC as preservatives is most important ingredient in liquid dosage form in order to maintain their shelf life.

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