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RP-HPLC Method development, validation for simultaneous estimation of Paracetamol and Mefenamic acid in bulk and its pharmaceutical formulations

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Abstract: **The quantitative analysis of Paracetamol and Mefenamic acid in pharmaceutical dosage form has been accomplished by the development of a straightforward, quick, accurate, sensitive, and repeatable reverse phase high performance liquid chromatography (RP-HPLC) method.**

Paracetamol and mefenamic acid were separated chromatographically on a Shimadzu LC-20AD using a SHISEIDO C18 (250 x 4.6 mm I.D., 5µm) column, mobile phase A (which contained ACN and methanol in a 90:10 ratio), and mobile phase B (which contained 0.1% orthophosphoric acid and ACN in a 35:65 ratio). 1.0 ml/min was the flow rate; a photodiode array detector operating at room temperature was used to detect absorbance at 213 and 230 nm. Paracetamol and mefenamic acid were found to have retention durations of 2.695 and 5.026 minutes, respectively. The correlation coefficients (r2) for paracetamol and mefenamic acid were 0.9935 and 0.9932, respectively, and linearity was found for both medications in the range of 3.25–16.25µg/ml and 5–25µg/ml. The percentage RSDs for paracetamol and mefenamic acid, respectively, were determined to be 10.73 and 5.45.

According to intra- and inter-day accuracy assessments, the relative standard deviation (%RSD) of the suggested approaches was less than the upper limit of 2.0. In compliance with ICH guidelines, the suggested approach was validated. The technique for quantitative analysis of Paracetamol and Mefenamic acid in bulk drugs and pharmaceutical formulations was determined to be straightforward, affordable, appropriate, precise, accurate, and robust.

I. INTRODUCTION

One of the most effective analytical methods for separating, identifying, and quantifying chemicals in chemical mixtures is High Performance Liquid Chromatography (HPLC). The ability of HPLC to analyze both volatile and non-volatile materials is one of its main benefits, which makes it a popular option for a variety of industries, including food production, medicines, medical research, and drug testing.

Fig:1 HPLC Instrument

A technique for separating components in a liquid mixture is called High Performance Liquid Chromatography (HPLC). After passing through a column filled with a separation medium (stationary phase), a liquid sample is injected into a stream of solvent (mobile phase). Differential migration causes the sample components to split from one another as they pass through the column.

PRINCIPLE-

A process of mass transfer that involves adsorption and/or partitioning is chromatography. As previously stated, HPLC uses pumps to move a sample combination and a pressurized liquid through an adsorbent-filled column, separating the sample's constituent parts. Usually composed of solid particles (such silica, polymers, etc.) that range in size from 1.5 to 50 µm, the adsorbent, the column's active component, can be coupled to a variety of reagents.[1][2]

Because of their varying degrees of contact with the adsorbent particles, the components of the sample mixture are separated from one another. The pressurized liquid is known as a "mobile phase" and is usually a combination of solvents (such as water, buffers, acetonitrile, and/or methanol?). Because they affect how the components of the sample interact with the adsorbent, its composition and temperature have a significant impact on the separation process.[3]

Instrumentation of HPLC

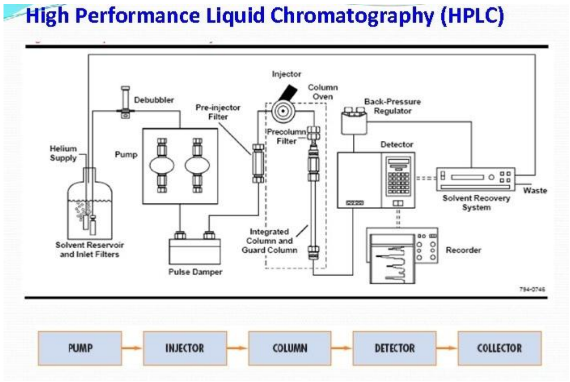


Fig:2 Instrumentation of HPLC

The Pump: The pump creates an eluent flow from the solvent reservoir into the liquid chromatography system by positioning itself in the uppermost stream.

Degasser: It's possible that the eluent used for LC analysis contains gases that are invisible to the human eye, including oxygen.

An injector: The pump is positioned adjacent to an injector.

The most straightforward technique involves introducing the sample into the eluent flow using a syringe.

A column: Inside the column, the separation is carried out.
In place of glass columns, the more modern ones are frequently manufactured in stainless steel housings.
The detector: The analytes are separated within the column, and the separation is seen using a detector. When there is no analyte present, the eluent's composition remains constant.

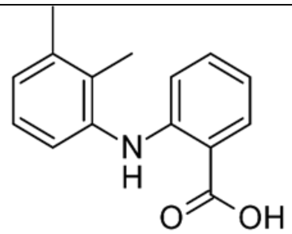
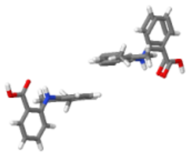
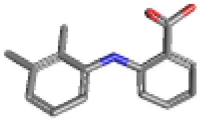
Recorder: Data processors come in a variety of forms, ranging from basic systems with built-in printers and word processors to those with software.

WORKING –

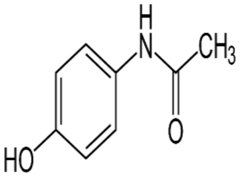
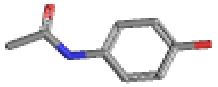
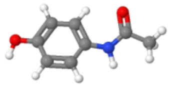
A flow-cell inside the detector receives the pumps' intended mobile phase flow and composition after passing through the stationary phase inside the column. The detector allows for quantitative examination of the sample components by producing a signal proportional to the amount of sample component emerging from the column. Additionally, the detector records the retention time, or the time of emergence, which is used for the component's initial identification. Additionally, more sophisticated detectors offer further details unique to the analyte's properties, like its mass or UV-VIS spectra, which might reveal details about its structural traits. These detectors are frequently used, including mass spectrometry detectors, photodiode array (PDA)/diode array detectors, and UV/Vis detectors.

DRUG PROFILE

MEFENAMIC ACID

structure	  
molecular formula	$C_{15}H_{15}No_2$
synonyms	mefenamic acid 61-68-7 ponstel 2-[(2,*3-dimethylphenyl)amino]benzoic* acid mephenamic acid
molecular weight	241.28 g/mol
description -	An aminobenzoic acid, mefenamic acid is an anthranilic acid in which a 2,3-dimethylphenyl group has taken the place of one of the hydrogen atoms bonded to the nitrogen.
physical description	solid
color	white to off-white
form	crystalline powder
melting point	230-231 °c
solubility	• >36.2 [ug/ml] (the average findings at pH 7.4) (20 mg/l) at 30 °C • White powder; water solubility: more than 5 g/100 ml; sodium salt • 1.37e-02 g/l
dissociation constants	acidic Pka 4.2 ,4.5
uv spectra	max absorption (0.1 n NaOH): 285 nm, 340 nm
pharmacological classes	nonsteroidal anti-inflammatory drug; anti-inflammatory agents, non-steroidal [cs]; cyclooxygenase inhibitors [moa]
methods of manufacturing	made by condensation of 2,3-dimethylaniline with o-chloro benzoic acid, catalyzed by copper.

PARACETAMOL

Structure	2D   3D  crystal
Molecular Formula	• $C_8H_9NO_2$ • $HOC_6H_4NHCOCH_3$
Synonyms	• acetaminophen • Paracetamol • 4-Acetamidophenol
Molecular Weight	151.16 g/mol
Description	The white, crystalline solid 4-hydroxyacetanilide has no smell. bitter flavour Paracetamol belongs to the 4-aminophenol class of phenols, which are characterized by the substitution of an acetyl group for one of the hydrogen atoms that were previously linked to the amino group.
Physical Description	Solid

Color	White
Form	Large monoclinic prism from water
Melting Point	336 to 342 °F
Solubility	• <22.7 [ug/mL] • At 72°F, 1 to 5 mg/mL; very barely soluble in cold water, but more soluble in hot water 14,000 mg/L in water at 25 °C; very weakly soluble in cold water; soluble in boiling water.
Dissociation Constants	pKa = 9.38
UV Spectra	UV max (ethanol): 250 nm
Pharmacological Class	Antipyretics, analgesics, Non-Narcotic
Methods of Manufacturing	Heating with a solution of glacial acetic acid, acetic anhydride reduces p-nitrophenol, acetylates the resultant p-aminophenol. By recrystallizing the crude product from an ethanol-water mixture

MATERIALS-

S. No.	Chemicals/Standards and reagents	Grade	Company
1	Orthophosphoric acid	HPLC	Finar
2	Acetonitrile	HPLC	Qualigens
3	Water	HPLC	Qualigens
4	Methanol	HPLC	Qualigens
5	Mefenamic acid	STD DRUGS	-
6	Paracetamol	STD DRUGS	-
7	Mefenamic acid and Paracetamol suspension		purchased from pharmacy Mankind

Table. 23: Materials and Chemicals

INSTRUMENTS:

UV-3092 LABINDIA double beam with UV win software, UV-Visible Spectrophotometer with 1 cm matched quartz cells. UFLC SHIMADZU Model: LC-20AD, UV-Visible dual absorbance detector with a manual injector. The output signal was monitored and integrated using Lab solutions software. A SHISEIDO C_{18} (250 x 4.6 mm I.D,5µm) column is used for separation.

S. No.	Equipment	Model	Company
1	Electronic balance	Ax200	SHIMADZU
2	Sonicator	D_{10}/IH	LOBA LIFE
3	P^H meter	7114	SYSTRONICS
4	Filter paper (0.45µm)	-	SARTORIOUS BIOLABS
5	Electronic balance	BL-2200H, D455202728	SHIMADZU

Table. 22: List of equipments

Preparation of standard stock solution

- Accurately weigh and transfer 10 mg of Paracetamol, 10 mg of Mefenamic acid working standard into a 10 ml clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (10ppm of Paracetamol, 10ppm of Mefenamic acid)

Sample Solution Preparation:

- The 5ml 0f suspension formulation, equivalent to 125 mg of PCT and 50 mg of MEF ACID was precisely pipetted out, followed by the formulation being transferred into a clean dry 100 ml volumetric flask. Then it was dissolved in equivalent ratio of two mobile phases and sonicated for 15 minutes for the complete dissolution of drugs. The solution was centrifuged up to 10 minutes at 3000 RPM and the above supernatant was pipetted out. The supernatant liquid was filtered by using 0.22µ filter.

Preparation of Mobile Phase:

Mobile phase A was prepared by mixing ACN and Methanol taken in the ratio 90:10. Mobile phase B was prepared by mixing 0.1% Ortho Phosphoric Acid and ACN taken in the ratio 35:65. It was filtered through 0.45µ membrane filter to remove the impurities which may interfere in the final chromatogram and sonicated for 5 minutes.

Method Validation

The established method in this study was validated following the quality guidelines of the international conference on harmonization, ICH Q2 (R1). The parameters are system suitability, robustness, linearity, and precision were studied by using the developed method. The system suitability of the method was assessed by injecting a stock solution containing 10 ppm PCT and 10 ppm MEF ACID six times into the HPLC system. The tailing factor, theoretical plate count, and resolution for the peaks were observed in chromatogram tables.

RESULTS AND DISCUSSION

A. Method development and optimization:

The choice of the detection wavelength was based on the scanned absorption spectrum of Paracetamol and Mefenamic acid 10mg of drugs were dissolved in 10ml of Acetonitrile and Methanol (90:10) and Orthophosphoric acid and Acetonitrile (35:65) separately. The UV spectrum of Paracetamol and Mefenamic acid was separately scanned in the wave length range 200-400 nm. After correlation of the spectrum 213nm and 230nm wavelength was selected for analysis.

Trails were performed using different columns (s polar 18, phenomenex, capcell PAK c18). The drugs were eluted at a flow rate of 1.0 ml/min using a mobile phase consisting of (Acetonitrile and Methanol) in the ratio of 90: 10 v/v and (OrthoPhosphoric Acid and Acetonitrile) in the ratio of 35: 65 v/v respectively. The retention times for Paracetamol and Mefenamic acid were found to be 2.695 and 5.026 min respectively.

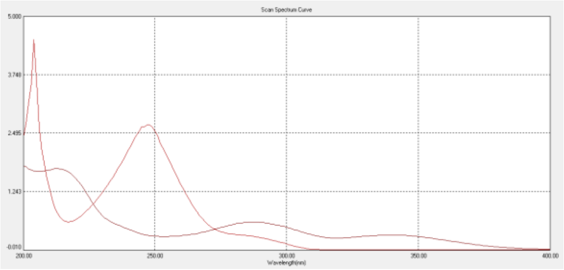


Fig 3: UV Overlay spectrum of Paracetamol and Mefenamic acid

B.System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

10µl of solution was injected in 6 replicates run into the HPLC system and examined with an emphasis on retention time and their standard deviations and relative standard deviations was calculated.

S.No	Parameter	Paracetamol	Mefenamic acid
1	Retention time	2.695	5.026
2	Plate count	4570	10235
3	Tailing factor	1.189	1.000
4	Resolution	-	13.050
5	%RSD	10.73697361	5.453053801

Acceptance Criteria: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

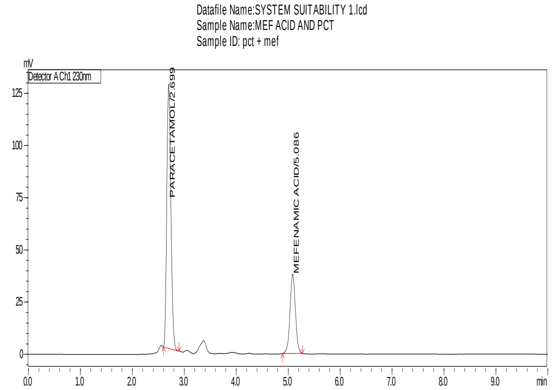


Fig 4: chromatogram of

A.Linearity: Linearity was established over the range of 3.25 -16.25µg/ml for Paracetamol and 5 -25µg/ml for Mefenamic acid using the weighted least square regression analysis and the results were shown in table 2, linearity graphs with time on x-axis and concentration on y-axis were down as fig 5a and 5b.

Fig 2a: Linearity plot of Paracetamol

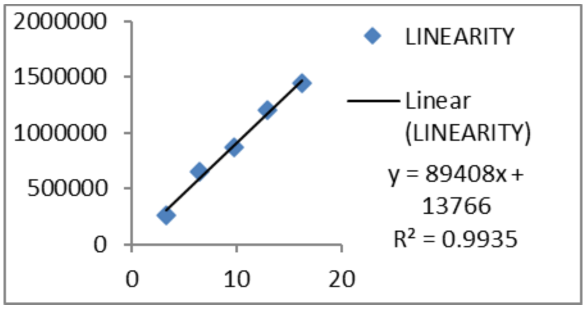
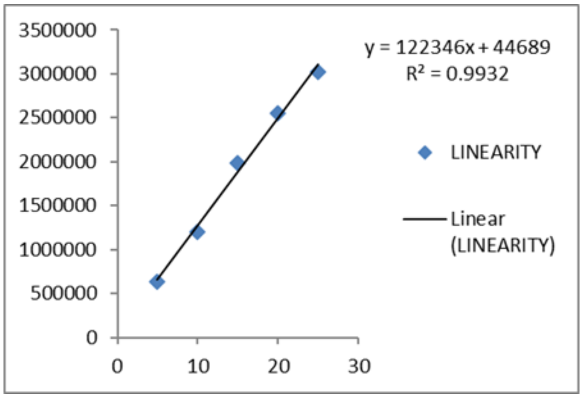


Fig 2b: Linearity plot of Mefenamic Acid

S.NO	Paracetamol		Mefenamic acid	
	Conc .(µg/ ml)	Peak area	Con c.(µ g/ml)	Peak area
1	3.25	265080.5	5	637767.5
2	6.5	646029.5	10	1202367
3	9.75	874962	15	1988120
4	13	1200823.5	20	2547069
5	16.25	1440557	25	3024063

Table 2. Linearity of Paracetamol and Mefenamic Acid



Regression equation	y = 89408x + 13766	y = 122346x + 44689
Slope	89408	122346
Intercept	13766	44689
R2	0.9935	0.9932

PRECISION:

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 5.15 % and 2.71 % respectively for Paracetamol and Mefenamic acid. As the limit of Precision was less than “2” the system precision was passed in this method. System precision, Method precision and Intermediate precision for Paracetamol and Mefenamic acid were found to be in Acceptance limits .Results were showed in Tables of 4a,4b and 4c.

Intermediate precision (Day_ Day Precision):

MEFENAMIC ACID			
	LQC	MQC	HQC
DAY1	668169	1854779.333	3022556.67
DAY2	977763	9167151	3050101
DAY3	628308	1749481	3063635
AVG	758080	4257137.111	3045430.89
SD	191292.1593	4252522.69	20933.5794
RSD	25.23376943	99.89160741	0.6873766

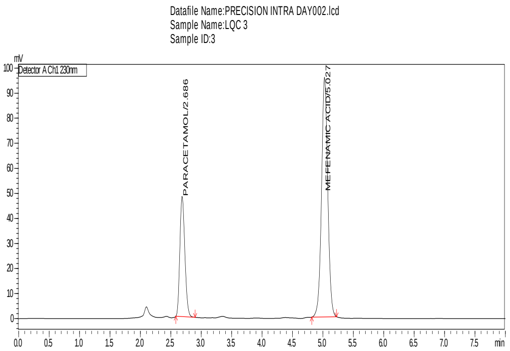
Table4c: Intermediate Precision (Day variation) for Paracetamol and Mefenamic acid

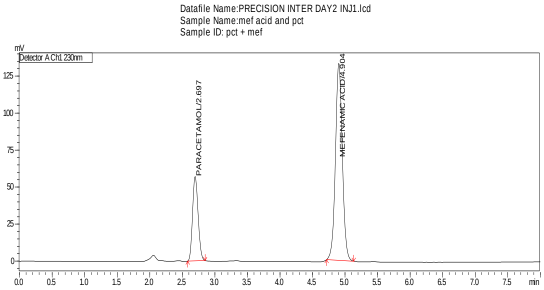
PARACETAMOL			
	LQC	MQC	HQC
DAY1	264515.3333	853972.6667	1297712
DAY2	338426	849819	1286927
DAY3	248244	735057	1314616
AVG	283728.4444	812949.5556	1299752
SD	48063.04204	67488.89461	13956.73
RSD	16.93980388	8.30173215	1.0738

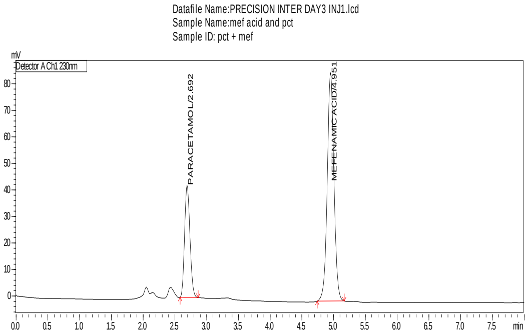
PCT			
SNO	LQC	MQC	HQC
1	237582	813853	1330683
2	280958	846988	1319626
3	275006	901077	1242828
AVG	264515.3333	853972.6667	1297712
SD	23514.03643	44029.48694	47851.67
RSD	8.889479538	5.155842647	3.687386
MEF ACID			
SNO	LQC	MQC	HQC
1	613410	1801802	3027910
2	710909	1901875	3057112
3	680188	1860661	2982648
AVG	668169	1854779.333	3022556.67
SD	49848.33017	50295.09752	37519.5343
RSD	7.46043743	2.711648583	1.24131781

Acceptance Criteria: The % RSD for the area of six standard injections results should not be more than 2%

Parameter	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (mL/min)	Less flow (0.9ml)	2.980	854334	1.172	4582
	Actual (1ml)	2.688	682650	1.184	4462
Organic Phase change	Less Org (33:67)	2.694	831249	1.216	4524
	Actual (35:65)	2.690	698893	1.189	4570
	More Org (37:63)	2.691	873241	1.209	4695







Robustness:

Optimal chromatographic conditions were deliberately modified to test the robustness of the method; a ± 2% adjustment was made in mobile phase B ratio(Orthophosphoric acid: Methanol = 33:67 and 37:63) and flow rate 1ml/min. And robustness was also tested by adjusting the flow rate 0.9ml/min with actual ratio. Robustness assessment was done two times by using concentration of 9.75µg/ml of PCT and 15µg/ml of Mef acid.

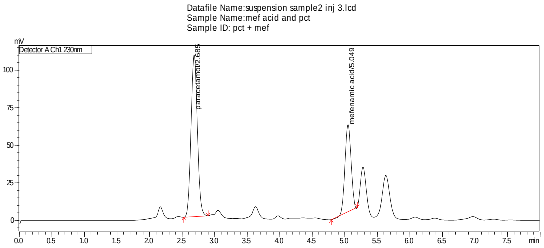
Table 6a: Robustness results of Paracetamol

Parameter	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (mL/min)	Less flow (0.9ml)	5.582	2063036	1.026	10909
	Actual (1ml)	5.030	247656	1.042	10835
Organic Phase change	Less Org (33:67)	4.931	1939544	1.037	9749
	Actual (35:65)	5.026	278878	1.000	10235
	More Org (37:63)	5.245	2037112	1.009	9801

Table 6b: Robustness results of Mefenamic acid

	Mef acid	PCT
y	107727	1152649
m	12234	89408
c	44689	13766
y-c	63038	1138883
y-c/m	5.152689227	12.73804358
conc	5	12.5
recovery	103.0537845	101.9043486

Assay using Formulation (Suspension of PCT and MEF Acid) Take 5ml of Nobel- Plus suspension containing 125mg of PCT and 50mg of MEF Acid in 100ml volumetric flask. Equal proportions of mobile phase was taken upto 50ml and sonicated for 15min.Then make up it upto 100ml.transfer 1ml from above stock solution to 10ml volumetric flask and make up to produce 100 µg/ml and repeat the same to produce 10 µg/ml. Centrifuge the above solution at 3000 RPM for 5min.Collect the supernatant liquid and filter it by using 0.22µ filter. Inject into the HPLC.



Acceptance Criteria: The percentage purity should be 95% to 105%.

CONCLUSION

The HPLC approach that was created for the measurement of specific pharmaceuticals is straightforward, quick, accurate, precise, reliable, and cost-effective. The solvents and mobile phase are inexpensive, dependable, sensitive, and time-efficient to prepare.

It is concluded that the short and straightforward suggested methods are the most helpful for analysis purposes because the system validation parameters such as linearity, precision, robustness, assay of the HPLC method used for estimation of selected drugs in pure have also demonstrated satisfactory, accurate, and reproducible results (without any interference of excipients).

Assay is performed for the Nobel- Plus suspension and the produced results are satisfactory.

Thus, these can be applied to routinely analyze Paracetamol and mefenamic acid.

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