



HPLC Quantitative Resolution of Acetaminophen, Dextromethorphan Hydrobromide, and Phenylephrine Hydrochloride in Powder Dosage Form by Chemometric Analysis Method

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ABSTRACT: A new simple, precise, accurate and selective RP-HPLC method has been developed and validated for stability indicating RP-HPLC method for simultaneous estimation of Acetaminophen, Dextromethorphan hydrobromide and Phenylephrine hydrochloride in powder dosage form by chemometric analysis methods. Two chemometric methods of PCA (principal component analysis), PLS (principle least square analysis) were applied for simultaneous estimation of Acetaminophen, Dextromethorphan hydrobromide and phenylephrine hydrochloride in powder dosage form. The chemometric applications were performed by using the UNSCRAMBLER software. Partial least square (PLS), principal component analysis (PCA) methods do not need any prior graphical treatment of the overlapping spectra of three drugs in a mixture. The method was carried out on a LunaC18, 250mm x 4.6mm, 5 μ m column with a mobile phase consisting of acetonitrile and buffer in the ratio of (20:80 v/v) and flow rate of 1ml/min. The detection was carried out at 210nm. The retention time for Acetaminophen, Dextromethorphan hydrobromide and Phenylephrine hydrochloride were found to be 4.22, 6.21 and 2.46mins respectively. The method was validated according to ICH guidelines for specificity, LOD, LOQ, precision, accuracy, linearity and robustness. The method showed good reproducibility and the recovery with %RSD less than 2. So the proposed method was found to be simple, specific, precise, accurate and linear. These two chemometric techniques in this study has supported to the HPLC method applied to the marketed dosage form for quantitative resolution of Acetaminophen, Dextromethorphan hydrobromide and Phenylephrine hydrochloride. © 2020 iGlobal Research and Publishing Foundation. All rights reserved.

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INTRODUCTION

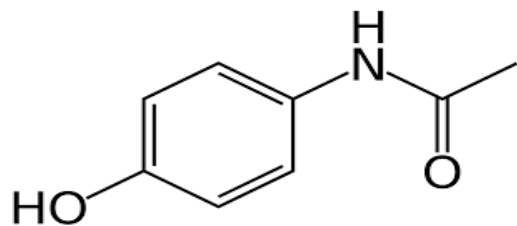
Data analysis plays a major role in assuring the quality of the bulk drug and pharmaceutical preparations which contributes to safety issue. Standard analytical procedure for the determination of newer drugs or formulation may not be available in pharmacopeias. Therefore, it is essential to develop the chemometric assisted RP-HPLC methods to provide a rapid quantitative analysis of pharmaceutical properties of intermediate and finished dosage forms. The chemometric analytical methods are accurate precise, specific, linear, simple and rapid. Many spectrophotometric and chromatographic methods have been reported for the determination of Acetaminophen, Dextromethorphan

hydrobromide and Phenylephrine hydrochloride in pharmaceutical dosage forms.

In some of these methods the data analysis is time consuming and lengthy process. Chemometric assisted RP-HPLC techniques involve the applying of methods like PCA, PLS to redundancy of noise and to determine the correlation between variables, covariance between different sets of variables.

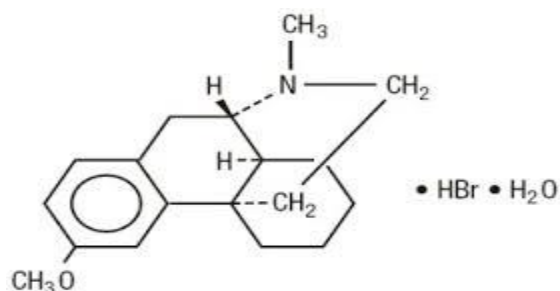
The acetaminophen is used as Analgesic and antipyretic, it acts by the Inhibition of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2. Because of its selectivity for COX-2 it does not significantly inhibit the production of the pro-clotting thromboxanes. While it

has analgesic and antipyretic properties comparable to those of aspirin or other NSAIDs, its peripheral anti-inflammatory activity is usually limited by several factors, one of which is the high level of peroxides present in inflammatory lesions [1].



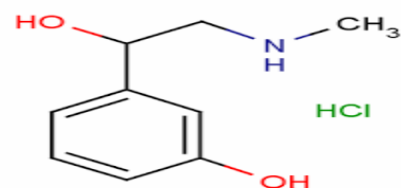
Structure of acetaminophen

Dextromethorphan is a anti tussive or non narcotic agent. The DXM is also used recreationally. When exceeding label-specified maximum dosages, dextromethorphan acts as a dissociative anesthetic. Its mechanism of action is via multiple effects, including actions as a nonselective serotonin reuptake inhibitor and a sigma-1 receptor agonist [2].



Structure of Dextromethorphan hydrobromide

The phenylephrine hydrochloride is a Adrenergic alpha-Agonists Nasal Decongestant. Phenylephrine acts predominantly by a direct effect on alpha-adrenergic receptors. In therapeutic doses, the drug has no substantial stimulant effect on the beta-adrenergic receptors of the heart (beta1-adrenergic receptors) but substantial activation of these receptors may occur when larger doses are given. Phenylephrine does not stimulate beta-adrenergic receptors of the bronchi or peripheral blood vessels (beta2-adrenergic receptors). It is believed that alpha-adrenergic effects result from the inhibition of the production of cyclic adenosine-3',5'-monophosphate (cAMP) by inhibition of the enzyme adenylcyclase, whereas beta-adrenergic effects result from stimulation of adenylcyclase activity. Phenylephrine also has an indirect effect by releasing norepinephrine from its storage sites [3,4].



Structure of phenylephrine hydrochloride

The Literature survey reveals that few HPLC methods [5-7], and uv-spectroscopy [8], ultra performance liquid chromatography [9] in combinations are available for simultaneous estimation. Hence a chemometric HPLC method has been proposed in this present research.

MATERIALS AND METHODS

Optimized chromatographic conditions

The developed RP-HPLC method for estimation of Acetaminophen, Dextromethorphan hydrobromide and Phenylephrine hydrochloride was carried out on Luna C18,(250mm x 4.6mm, 5μm) column using mobile phase composition of mixture of Acetonitrile and Buffer in the ratio of (20:80V/V), flow rate of 1.0 ml /min at 210 nm. The software used is Empower version 2.0, unscramble-X.

Preparation of buffer solution

Mixed 1ml of orthophosphoric acid in 1 lt water and filtered through 0.45 μm nylon membrane filter. Mobile phase : A mixture of Water and Acetonitrile in the ratio of 30:70%v/v was sonicated to degas and filtered through 0.45μm nylon membrane filter.

Preparation of standard stock solution

Acetaminophen

Weighed accurately about 5.6mg of Acetaminophen working standard into a 100 mL volumetric flask. Added 70 mL of diluent sonicate to dissolved and diluted to volume with diluent.

Dextromethorphan HBr

Weighed accurately about 0.224mg of Dextromethorphan HBr working standard into a 100 mL volumetric flask. Added 70 mL of diluent, sonicate to dissolve and diluted to volume with diluent.

Phenylephrine HCl

Weighed accurately about 0.112mg of Phenylephrine HCl working standard into a 100 mL volumetric flask. Added 70 mL of diluent, sonicate to dissolve and diluted to volume with diluent. Further diluted each 5mL of Solution-A, B and C to 50 mL with the diluent

Preparation of standard stock solution

Weighed accurately about 500mg of phenylephrine HCl standard stock solution and 5.6 mg of Acetaminophen working standard and 1.12 mg of Dextromethorphan Hbr into a 100mL volumetric flask. Added 80mL of diluents and sonicated to dissolve and diluted to volume up to the mark with diluents. Further the above solution is made to 50mL with the Diluent.

Preparation of sample solution

Weighed accurately about 11.2mg of sample taken into a 100 mL volumetric flask. Added 70 mL of diluent, sonicated to dissolve and diluted to volume diluent. Further diluted 5 mL to 50 mL with the diluent. Filtered through 0.45 μ Nylon syringe filter.

Procedure

Injected 10 μ L of Standard preparation five times and Sample preparation in the Chromatograph. Recorded the chromatograms and measured the peak responses for PhenylephrineHCl, Acetaminophen and Dextromethorphan HBr. The System suitability parameters should be met. From the peak responses, calculated the content of

PhenylephrineHCl, Acetaminophen and Dextromethorphan HBr in the sample.

RESULTS AND DISCUSSION

The results are summarized in **Tables 1-3** and **figures 1-7** and are self explanatory. The complexity of problems encountered in pharmaceutical analysis with the importance of achieving is the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs. Chemometric methods are less expensive methods and they do not require sophisticated instrumentation and any prior separation step. This can be considered a superiority of these chemometric techniques over HPLC. But they need software for resolution and determination of active ingredients in the mixtures. The chemometric methods proposed for PhenylephrineHCl, Acetaminophen and Dextromethorphan Hbr are very powerful methods for the simultaneous estimation or analysis of these three drugs in combined dosage form.

Table 1. Assay of PhenylephrineHCl, Acetaminophen and Dextromethorphan Hbr

| DRUG | Area | LABELED AMOUNT(mg) | AMOUNT PRESENT(mg) | % ASSAY |
|----------------------|---------|--------------------|--------------------|---------|
| Acetaminophen | 688760 | 500 | 496 | 100.7 |
| Dextromethorphan HBr | 2556977 | 20 | 20.4 | 100.4 |
| Phenylephrine HCl | 496818 | 10 | 10.2 | 100.6 |

Table 2. Validation parameters of proposed method

| Parameter | Acceptance criteria | Acetaminophen | Dextromethorphan hydrobromide | Phenylephrine hydrochloride |
|--------------------------------|--|---|---|---|
| Linearity range | Correlation coefficient | 0.998 | 0.998 | 0.998 |
| Correlation coefficient | $r^2 > 0.999$ | | | |
| System precision | %RSD < 2% | 0.754 | 0.441 | 0.558 |
| Intermediate precision | %RSD < 2% | 0.682 | 0.539 | 1.127 |
| Method precision | %RSD < 2% | 0.899 | 0.813 | 1.215 |
| Accuracy | %Recovery 98-102% (Individual) | 100.5 | 100.5 | 100.7 |
| Solution stability | > 12h | Stable upto 24hrs % RSD= 1.48 | Stable upto 24hrs % RSD= 1.35 | Stable upto 24hrs % RSD= 1.29 |
| Robustness | %RSD NMT 2% in modified condition flow minus flow plus Organic plus organic minus Wavelength plus Wave length minus | Complies %RSD= %RSD=0.650 %RSD=0.244 %RSD=0.218 %RSD=0.127 %RSD=1.195 %RSD=0.479 | Complies %RSD= %RSD=1.061 %RSD=0.479 %RSD= 0.166 %RSD=0.221 %RSD=0.160 %RSD=0.364 | Complies %RSD= %RSD=1.04 %RSD=0.309 %RSD=0.532 %RSD=0.501 %RSD=1.607 %RSD=0.213 |

Table 3. Linearity data of acetaminophen, dextromethorphan Hbr, phenylephrine hydrochloride

| Linearity | Solution taken | Actominophen | | Dextromethorphan Hydrobromide | | phenylephrine hydrochloride | |
|------------|----------------|-----------------------|-------------|-------------------------------|-------------|-----------------------------|-------------|
| | | Concentration (µg/mL) | area counts | Concentration (µg/mL) | area counts | Concentration (µg/mL) | area counts |
| Linearity1 | 0.5 | 0.56 | 234513 | 0.02 | 51867 | 0.06 | 12944 |
| Linearity2 | 1 | 1.12 | 430692 | 0.07 | 164034 | 0.14 | 32683 |
| Linearity3 | 2.6 | 2.91 | 1135212 | 0.11 | 236192 | 0.28 | 61229 |
| Linearity4 | 5 | 5.60 | 2067474 | 0.22 | 475244 | 0.58 | 126035 |
| Linearity5 | 6.25 | 7.00 | 2583660 | 0.28 | 574228 | 0.67 | 139742 |
| Linearity6 | 7.5 | 8.40 | 2995395 | 0.32 | 651839 | 0.84 | 176453 |

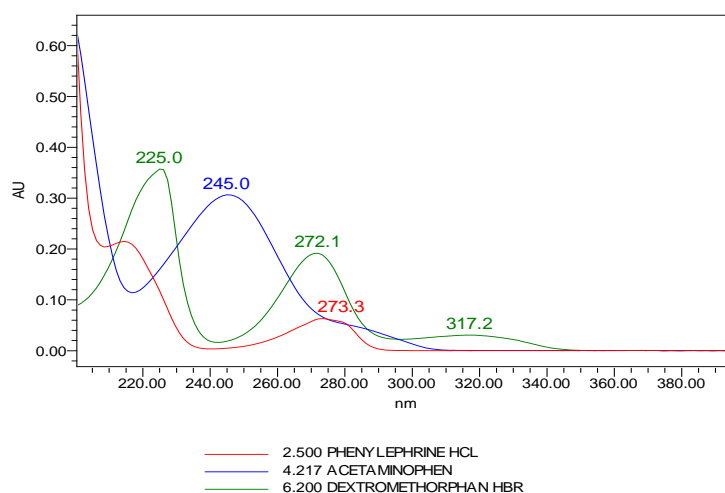


Figure 1. Overlay spectrum of phenylephrine hydrochloride, acetaminophen and dextromethorphan Hydro bromide

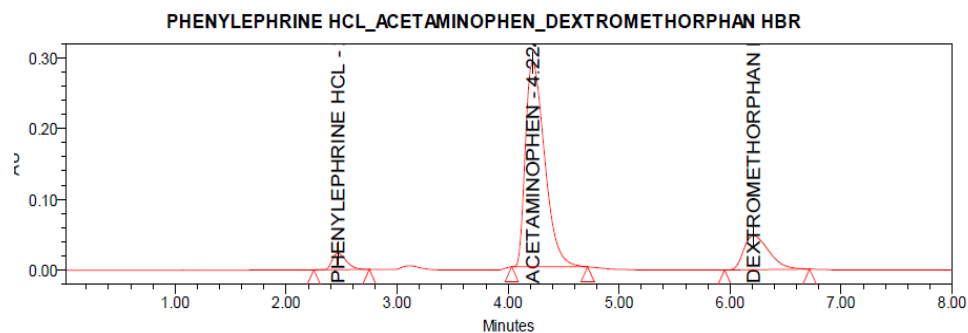


Figure 2. A representative chromatogram of Standard

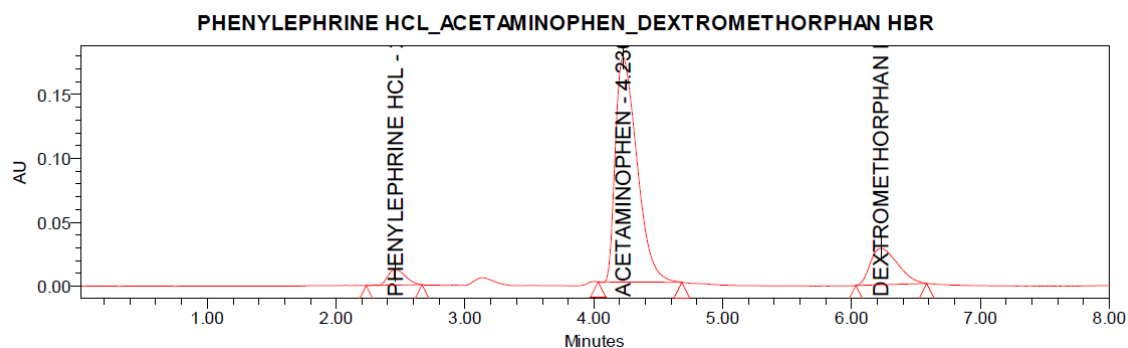


Figure 3. A representative chromatogram of Sample

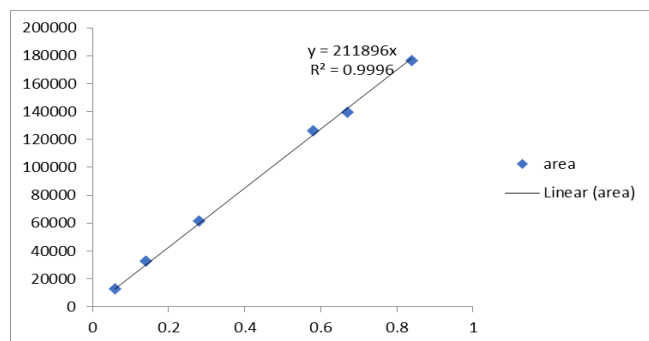


Figure 4. Linearity graph of Acetaminophen.

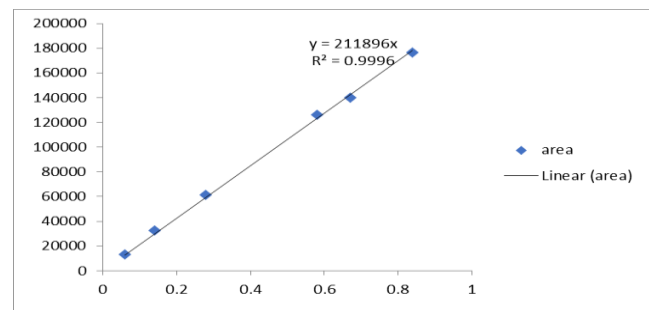


Figure 5. Linearity graph of Dextromethorphan HBr.

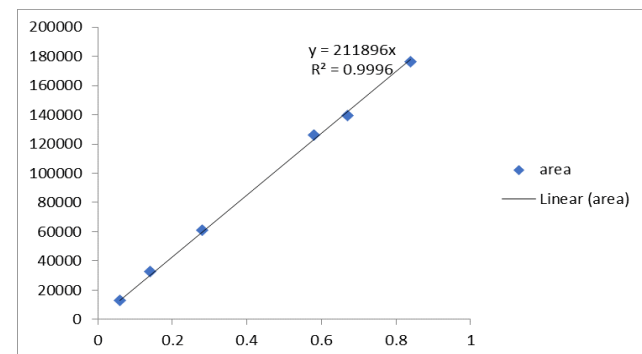


Figure 6. Linearity graph of Phenylephrine HCl.

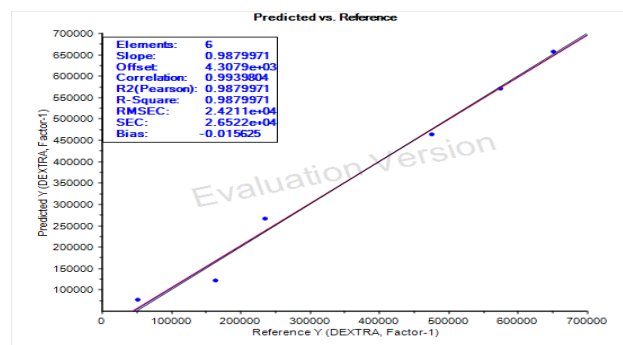


Figure 8. PLS Linearity spectral data of Dextromethorphan HBr

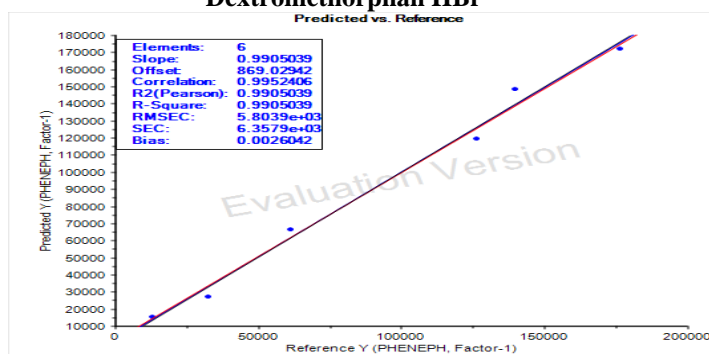


Figure 9. PLS Linearity spectral data of phenylephrine HCl

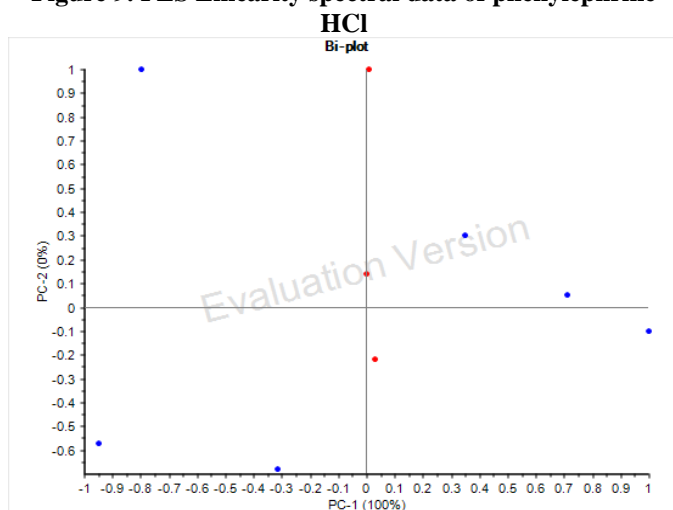


Figure 10. PCA Linearity spectral data of Acetaminophen, Dextromethorphan HBr and Phenylephrine HCl

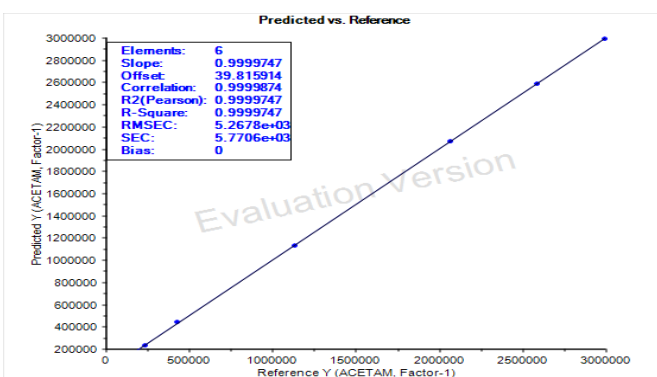


Figure 7. PLS Linearity spectral data of Acetaminophen

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DATA AVAILABILITY

Not Declared.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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