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# Novel Liquid Chromatographic Method for the Simultaneous Estimation of Dextromethorphan and Amylmetacresol

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**Abstract :** The aim of the present work is to develop a simple, rapid, accurate and precise reverse phase-high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of Dextromethorphan and Amylmetacresol and to validate as per international conference on harmonization (ICH) guidelines. The chromatographic separation was performed on Discovery C18 column (250 mm×4.6 mm, 5 µm), a mobile phase comprising of mixed phosphate buffer: acetonitrile (50:50) pumped at a flow rate of 1.0 ml/min and a detection wavelength of 220 nm using a PDA detector. The developed method resulted in elution of Dextromethorphan at 4.120±0.01 min and Amylmetacresol at 5.300±0.01 min. The calibration curves were linear ($r^2$=0.999) in the concentration range of 2.5-7.5 µg/ml and 0.3-0.9 µg/ml for Dextromethorphan and Amylmetacresol respectively. The percentage recoveries were found to be 99.29-100.46 % for Dextromethorphan and 99.80-101.36 % for Amylmetacresol. The LOD was found to be 0.29 µg/ml and 0.86 µg/ml for Dextromethorphan and Amylmetacresol respectively. LOQ was found to be 0.05 µg/ml and 0.15 µg/ml for Dextromethorphan and Amylmetacresol respectively.A simple, rapid, accurate and precise RP-HPLC method was developed for simultaneous estimation of DM and AMC in bulk and pharmaceutical formulation and validated as per ICH guidelines. Hence the method holds good for the routine quality control of DM and AMC in bulk and pharmaceutical formulation.
**Keywords :** Dextromethorphan, Amylmetacresol, RP-HPLC, method development, validation.

## Introduction:

Dextromethorphan (fig. 1) is an opioid like drug that acts as antagonist to the N-methyl-D-aspartaseglutamatergic receptor. It is an agonist to the opioid sigma 1 and sigma 2 receptors and targets the serotonin reuptake pump. It is chemically 3-methoxy-17-methylmorphinan. It is one of the widely used antitussives.

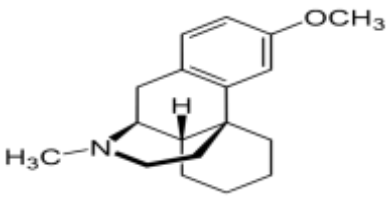


**Fig. 1: Structure of Dextromethorphan**

Amylmetacresol(fig. 2) is a phenolic antiseptic. It is used mainly as an ingredient in lozenges for the treatment of minor infections of the mouth and throat by killing the bacteria associated with mouth and throat infections.It is chemically 5-methyl-2-pentylphenol.A combination of Dextromethorphan and Amylmetacresol is mainly used in treatment of mouth and throat infections.

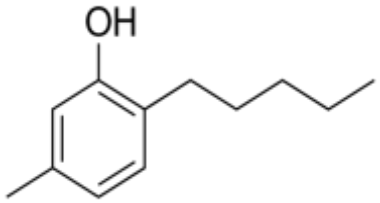


**Fig. 2: Structure of Amylmetacresol**

A detailed literature survey revealed that there were ultra violet(UV) spectrophotometric methods for estimation of Dextromethorphan with other combinations [1-2]. Various reverse phase high performance liquid chromatographicmethods (RP-HPLC) for the estimation of DM with other combinations [3-12]. But there was no RP-HPLC method yet reported for simultaneous estimation of Dextromethorphan and Amylmetacresol. Hence an attempt has been made to develop a rapid, accurate and precise HPLC method for simultaneous estimation of Dextromethorphan and Amylmetacresolin bulk and pharmaceutical dosage form. The developed method was validated as per ICH guidelines [13]. The validated method was used for the quantification of marketed formulation containing specified drugs.

## Experimental work:

### Chemicals and reagents

Pharmaceutical grade Dextromethorphan and Amylmetacresol were supplied as a gift sample by Spectrum Pharma Labs, Hyderabad and marketed formulation (TUSQ-D) was purchased from the local market. Orthophosphoric acid (OPA), acetonitrile and HPLC grade water were obtained from Merck Specialties Pvt. Ltd., Mumbai.

### Instrumentation

RP-HPLC Waters (e2695) system consisting of binary gradient pump with PDA detector and rheodyne injector with 20 µl fixed loop was used for injecting sample in this study. Empower software was employed in this method.

### Chromatographic conditions

The developed method used a reverse phase Discovery C18 column (250×4.6 mm, 5µm), a mobile phase of mixed phosphate buffer ($p^H$4.0): acetonitrile (50:50), flow rate of 1.0 ml/min and a detection wavelength of 220 nm.

### Preparation of mixed phosphate buffer

11.45 g of potassium dihydrogen phosphate ($KH_2PO_4$) and 28.8 g of disodium hydrogen orthophosphate ($Na_2HPO_4$) was accurately weighed and dissolved in water and volume was made up to 1000 ml with water. The $p^H$was adjusted to 4.0 by using orthophosphoric acid. The buffer was sonicated for 15 min and then filtered.

### Diluent

HPLC grade water and acetonitrile in the ratio 80:20 was used as diluent.

**Preparation of standard solutions [14]**

Standard stock solutions of Dextromethorphan and Amylmetacresol were prepared bydissolving5 mg of Dextromethorphan and 0.6 mg of Amylmetacresol working standards in sufficient diluent. After that, the solution was filtered and sonicated for 5 min and diluted to 100 ml with diluent. Further dilutions were prepared in 5 replicates of 5µg/ml of Dextromethorphan and 0.6 µg/ml of Amylmetacresol by adding 1 ml of the above stock solution was taken into 10 ml of diluent. This has been treated as 100 % target concentration.

**Preparation of sample solution**

20 lozenges were weighed, crushed and a powder equivalent to 5 mg of Dextromethorphan and 0.6 mg of Amylmetacresol working standards in sufficient diluent. After that, the solution was filtered and sonicated for 5 min and diluted to 100 ml with diluent. Further dilutions were prepared by adding 1 ml of the above stock solution was taken into 10 ml of diluent. This has been treated as 100 % target concentration.

## Results and Discussion:

**Method development**

Different chromatographic conditions were tried for better separation and resolution. Discovery (250×4.6 mm, 5 µm) column was found satisfactory. A number of solvents in the different ratio over a wide range of $p^H$ were tried, but didn't get satisfactory results. Repeated trials were performed to obtain good, sharp peak with an efficient resolution between two peaks of Dextromethorphan and Amylmetacresol on a C18 column in isocratic mode. The run time was good in isocratic trial with mobile phase consisting of mixed phosphate buffer ($p^H$4.0):acetonitrile (50:50) and Discovery C18 column (250×4.6 mm, 5µm), flow rate 1.0 ml/min, sample volume 20 µl and detection wavelength 220 nm gave the satisfactory results in terms of retention time, resolution, symmetry and sensitivity. A typical RP-HPLC chromatogram for Dextromethorphan and Amylmetacresolfrom standard preparation and pharmaceutical formulation was shown. (fig. 3 and 4).

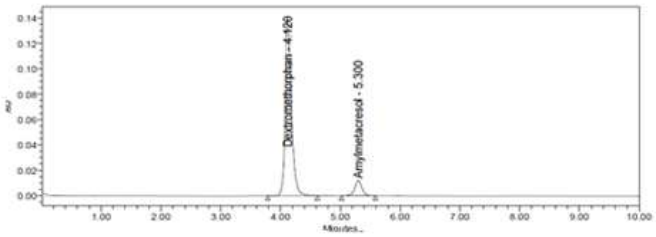


**Fig. 3: Standard chromatogram of Dextromethorphan and Amylmetacresol**

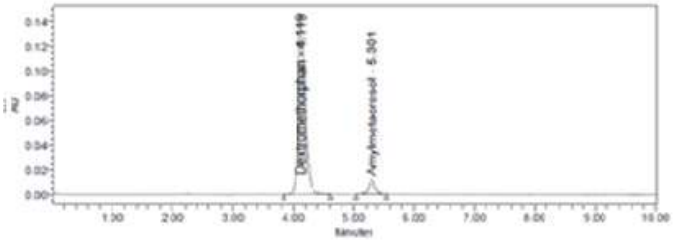


**Fig. 4: Sample chromatogram of Dextromethorphan and Amylmetacresol**

**Method validation**

The proposed analytical method was validated for system suitability, linearity, precision, accuracy, robustness, LOD and LOQ in accordance with ICH guidelines for analytical procedures Q2[R1].

**System suitability**

System suitability parameters were studied to verify the system performance. Standard solutions were prepared as per the test method and were injected six times into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. The system suitability parameters were tabulated in Table 1. All the parameters were found to be within the limits.

**Table 1: Results of system suitability studies**

| **Parameters** | **Acceptance limits** | **Dextromethorphan** | **Amylmetacresol** |
|---|---|---|---|
| Retention time* (min) | - | 4.120±0.01 | 5.300±0.01 |
| Resolution* | NLT 2 | - | 14.232±0.015 |
| Theoretical plates* | NLT 2000 | 8672±5 | 7583±3 |
| Tailing factor* | NMT 2 | 0.93±0.01 | 0.96±0.01 |

***=results of six determinations**

**Method precision**

The precision of the method was verified by method precision studies. The precision of the developed analytical method was carried out for same concentration level of standard solution. Six determinations were performed and were expressed in term of percentage relative standard deviation [% RSD]. The results of precision were tabulated in Table 2. Method precision % RSD values lower than 2% clearly assured that the developed method was found to be fairly precise and reproducible.

**Table 2: Results of method precision studies**

| **n** | **Dextromethorphan** | | **Amylmetacresol** | |
|---|---|---|---|---|
| | **Rt** | **Peak area** | **Rt** | **Peak area** |
| Injection 1 | 4.129 | 1006254 | 5.303 | 103682 |
| Injection 2 | 4.119 | 1005841 | 5.299 | 103474 |
| Injection 3 | 4.120 | 1013578 | 5.300 | 102143 |
| Injection 4 | 4.121 | 1019828 | 5.301 | 103267 |
| Injection 5 | 4.125 | 1005803 | 5.300 | 102604 |
| Injection 6 | 4.123 | 1017807 | 5.299 | 102984 |
| **Mean*± SD** | 1011519±6410.2 | | 103026±574.2 | |
| **% RSD**[#] | 0.63 | | 0.55 | |

**n=6 determinations, *= results of 6 observations, SD=Standard Deviation,**

**RSD=Relative Standard Deviation, [#]Acceptance criteria: <2**

**Linearity**

Linearity was evaluated by analysis of working standard solutions of Dextromethorphan and Amylmetacresol standard stock solution at five concentration levels from 50% to 150% of assay concentration. The peak area versus concentration data was treated by least square linear regression analysis (fig. 5 and 6). The results were tabulated in Table 3, have shown an excellent correlation between peak areas and concentration within the concentration range of 2.5-7.5 µg/ml for DM, 0.3-0.9 µg/ml for AMC. The correlation coefficients were found to be 0.999 for both the drugs, which meet the method validation acceptance criteria and hence the method was said to be linear for both the drugs at specified concentration range.

**Table 3: Linearity data for dextromethorphan and amylmetacresol**

| **Dextromethorphan** | | **Amylmetacresol** | |
|---|---|---|---|
| **Concentration (µg/ml)** | **Peak area** | **Concentration (µg/ml)** | **Peak area** |
| 2.50 | 524339 | 0.30 | 53176 |
| 3.75 | 743745 | 0.45 | 75355 |
| 5.00 | 1006324 | 0.60 | 101753 |
| 6.25 | 1257926 | 0.75 | 125155 |
| 7.50 | 1552138 | 0.90 | 147927 |
| **Slope**<br>**Correlation coefficient** | **20432**<br>**0.999** | **Slope**<br>**Correlation coefficient** | **16425**<br>**0.999** |

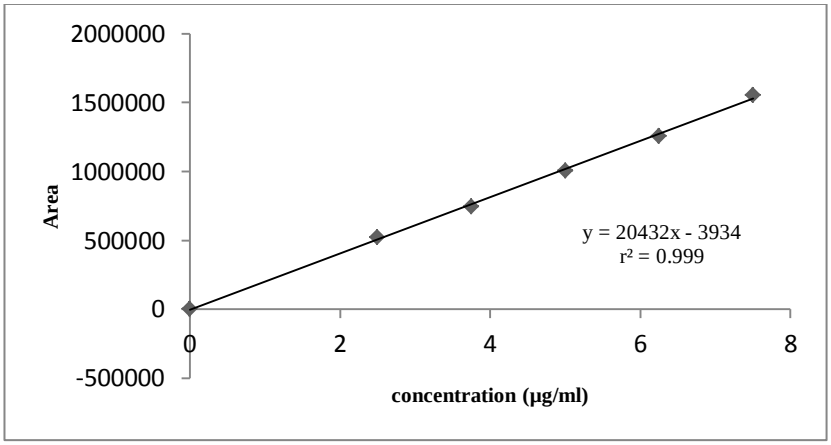


**Fig. 5: Linearity chart for Dextromethorphan**

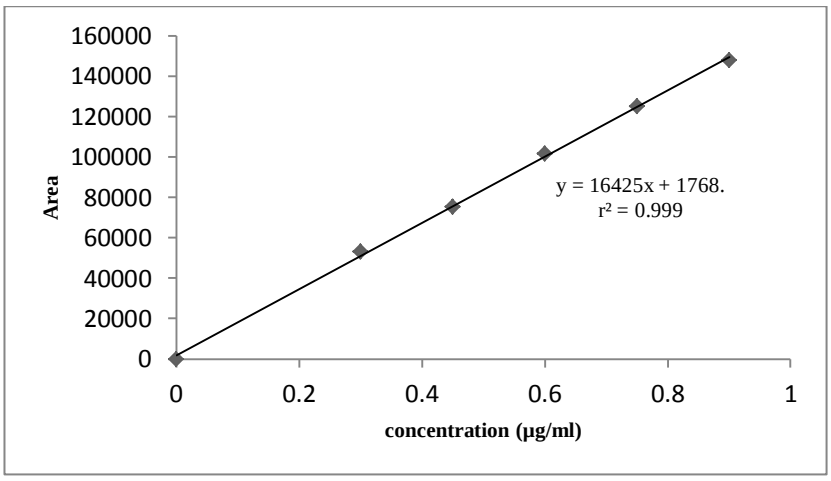


**Fig. 6: Linearity chart for amylmetacresol**

**Accuracy**

The accuracy of the method was determined by recovery studies, by determining % mean recovery of both the drugs at three different levels (50 %, 100 % and 150%). At each level, three determinations were performed. The percentage recovery and mean percentage recovery were calculated for the drug. The results were shown in table 4. The observed data were within the required range, which indicates good recovery values and hence the accuracy of the method developed.

**Table 4: Results of accuracy studies**

| Level (%) | Dextromethorphan | | Amylmetacresol | |
|---|---|---|---|---|
| | % Recovery | % Mean | % Recovery | % Mean |
| 50 | 100.1 | 100.46 | 100.0 | 99.80 |
| 50 | 100.8 | | 99.40 | |
| 50 | 100.5 | | 100.0 | |
| 100 | 100.2 | 100.23 | 101.2 | 100.23 |
| 100 | 100.0 | | 99.30 | |
| 100 | 100.0 | | 100.2 | |
| 150 | 99.10 | 99.29 | 101.3 | 101.36 |
| 150 | 99.49 | | 102.4 | |
| 150 | 99.30 | | 100.4 | |

**Robustness**

The robustness of the developed method was determined by altering the experimental conditions were deliberately, and the system suitability parameters were evaluated. The solutions were prepared as per the test method and injected at different variable conditions like flow rate (0.8 ml/min, 1.2 ml/min.) and wave length (218 nm, 222 nm), system suitability parameters were compared with that of method precision. The results were tabulated in table 5. At the flow rate of 1.0 ml/min shows, a sharp peak with good resolution and rest of the flow rates were found to be not satisfactory. Similarly at wave length of 220 nm all the parameters were found satisfactory when compared to rest of wave lengths. The method passed all system suitability parameters indicating that the method was robust.

**Table 5: Results of robustness studies**

| Parameter | Dextromethorphan | | Amylmetacresol | |
|---|---|---|---|---|
| | Plate count | Tailing | Plate count | Tailing |
| Less flow rate (0.8 ml/min) | 8143 | 1.736 | 8143 | 1.361 |
| More flow rate (1.2 ml/min) | 8621 | 1.533 | 8672 | 1.766 |
| Less wave length (218 nm) | 6188 | 0.936 | 8014 | 0.961 |
| High wave length (222 nm) | 5827 | 0.933 | 4749 | 0.966 |

**LOD and LOQ**

Limit of detection (LOD) which represents the concentration of the analyte at S/N ratio of 3:1and limit of quantitation (LOQ) at which S/N was 10:1 were determined experimentally for the proposed method and results were given in table 6.

**Table 6: Results of LOD and LOQ**

| Sample name | LOD (µg/ml) | LOQ (µg/ml) |
|---|---|---|
| Dextromethorphan | 0.29 | 0.86 |
| Amylmetacresol | 0.05 | 0.15 |

## Conclusion

The proposed method was simple, rapid, precise, accurate and robust. The developed method was also utilized for assay of commercial lozenges and obtained values are good agreement with their labeled claim. These advantageous encourage that the developed method can be utilized for routine quality control of specified combination in bulk and pharmaceutical formulation.

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## Conflict of Interests

Declared none

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