

Microemulsion High Performance Liquid Chromatography (MELC) Method Validation for the Quantification of Terbutaline in the Bricanyl Turbohaler®

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- *Introduction*

- Method Development

- Method Validation

- Conclusions



- Introduction

Method Development

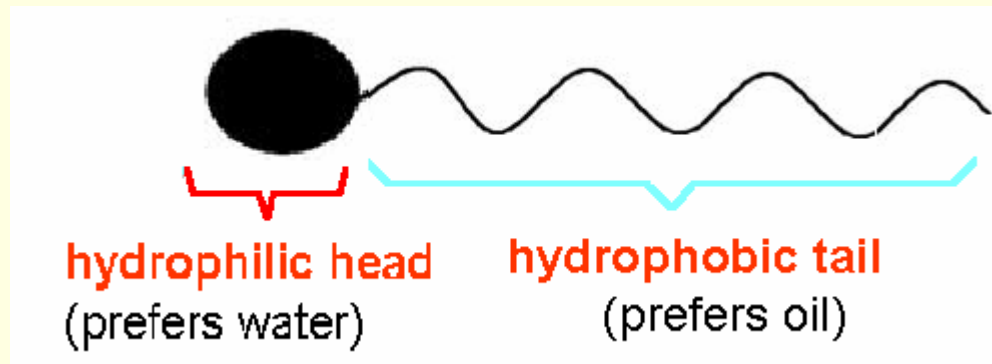
Method Validation

Conclusions



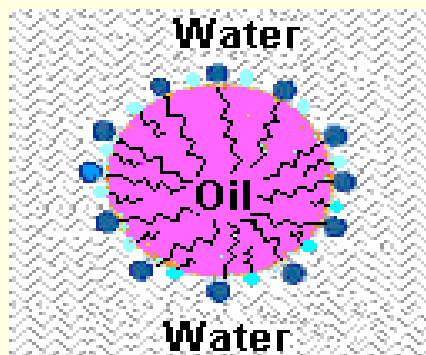
What is a Microemulsion ?

- ▶ Oil
- ▶ Water
- ▶ Surfactant
- ▶ Co surfactant

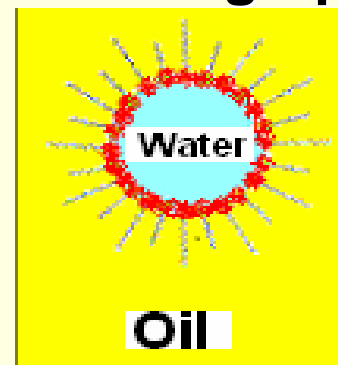


Types of Microemulsions:

- Water-in-oil: Normal-phase chromatography
- Oil –in-water : Reversed-phase chromatography



Oil-in-Water

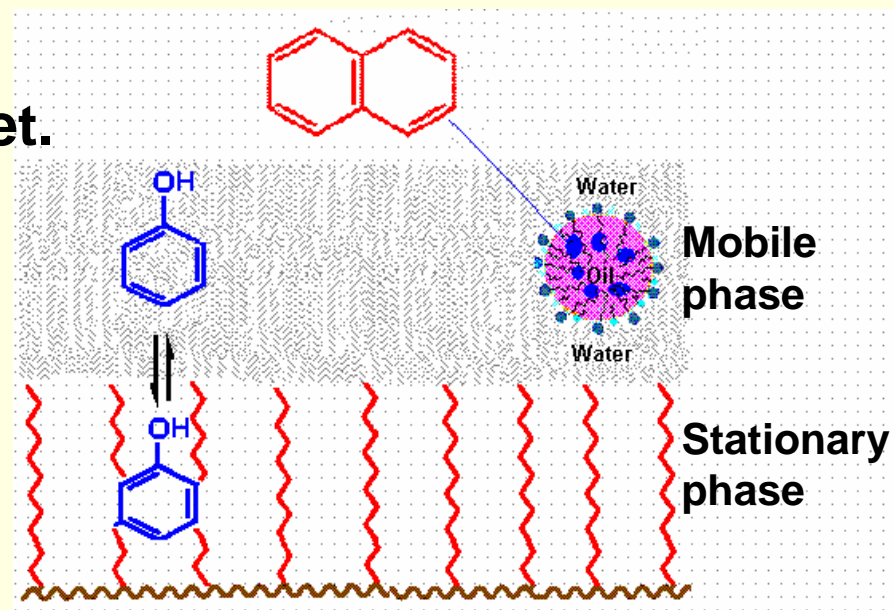


Water-in-oil

Oil –in-water microemulsion HPLC

Lipophilic compound:
favour inclusion in the oil droplet.

Hydrophilic compounds:
Their retention will mostly
controlled by the stationary
phase interactions.



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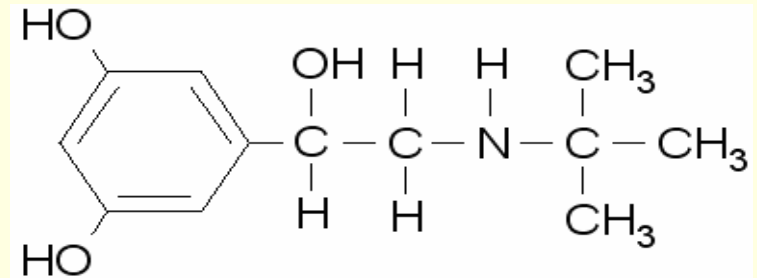
Conclusions



Oil –in-water microemulsion HPLC

Terbutaline

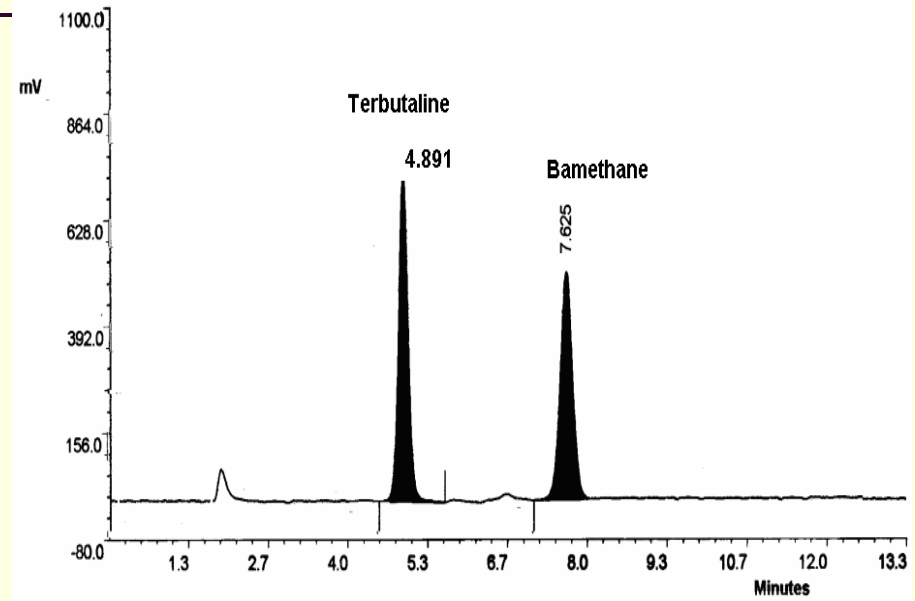
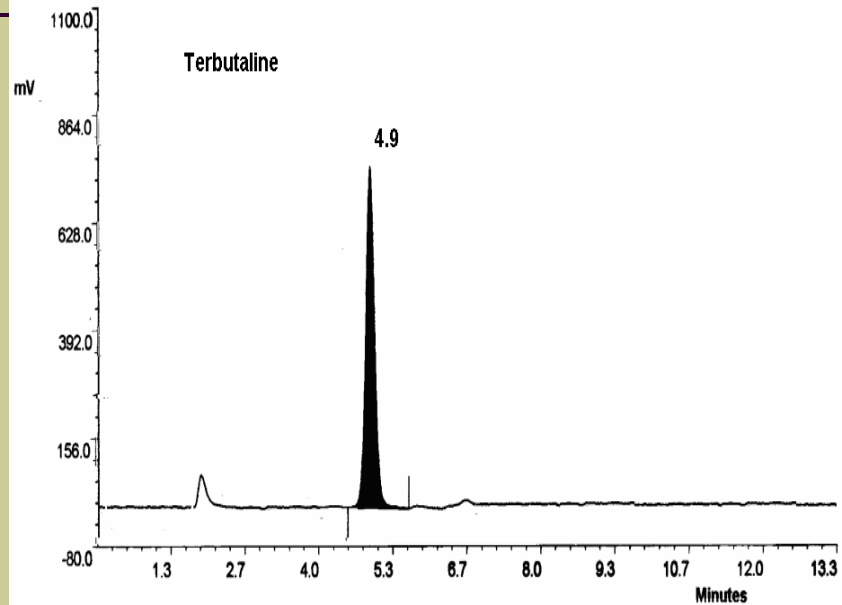
Short acting β_2 -agonist



Terbutaline is available in the following dosage form:

- **Tablets**
- **Solution**
- **Inhalers:**
 - **Dry powder inhalers (DPIs): Bricanyl Turbohaler**
 - **Metered dose inhalers (MDIs)**
 - **Nebuliser**

-Optimised Separation of Terbutaline and Bamethane in Aqueous



Conditions:

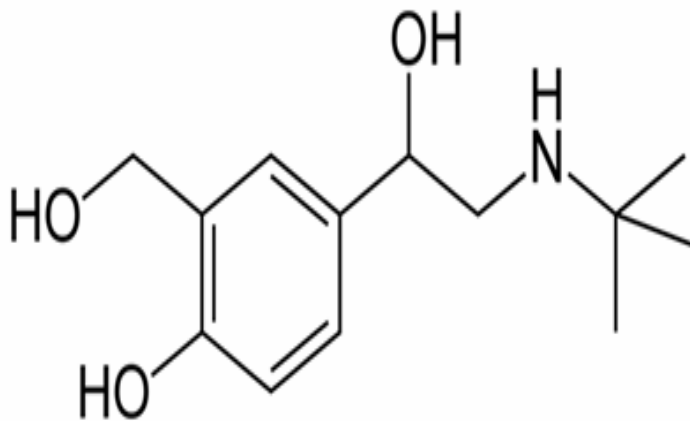
Mobile Phase: isocratic, 1.5%Brij35: 0.5% Ethyl acetate: 3%Butanol: 95% Phosphate buffer pH3.

Column: Hichrom ODS1, Wavelength: λ_{ex} 267nm λ_{em} 313nm: Room Temperature



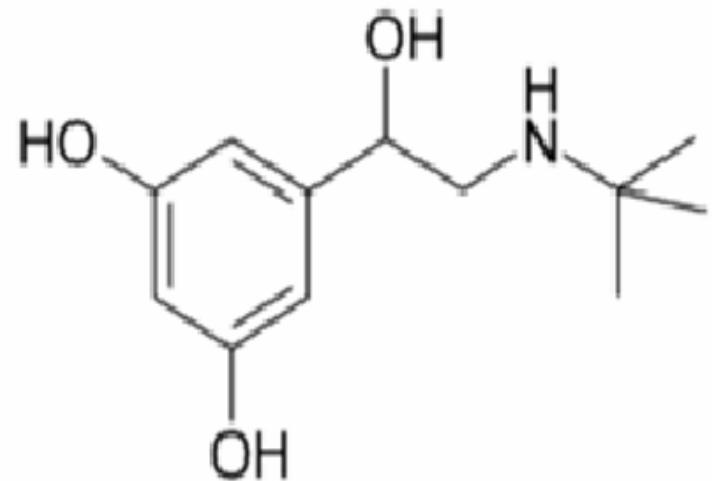
Similar Structures :

Salbutamol



1

Terbutaline

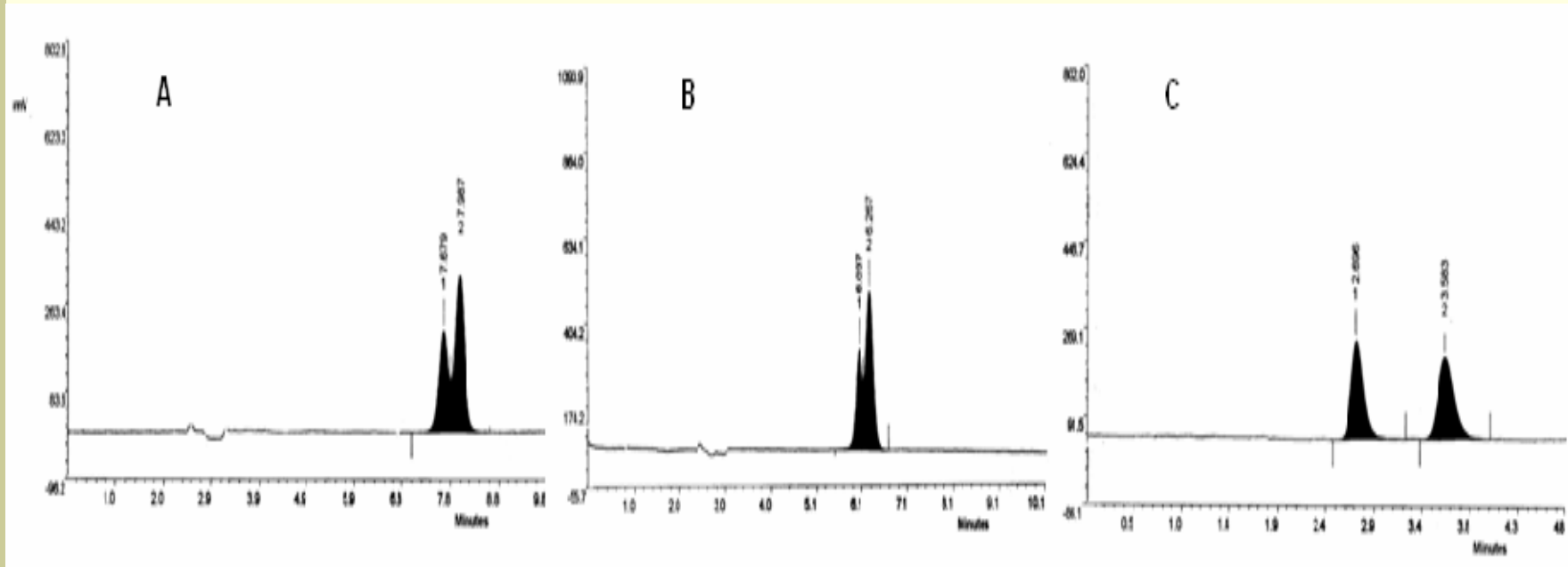


Comparison with other mobile phase

- ACN: Phosphate buffer

-Methanol: phosphate buffer

-MELC



A) ACN : 5mM Phosphate buffer (pH3) [25:75]

B) Methanol: 5mM Phosphate buffer (pH3) [50:50]

C) Microemulsion: Brij35 1.5%: Ethyl acetate 0.5%: Butanol 3% and Phosphate buffer 95%



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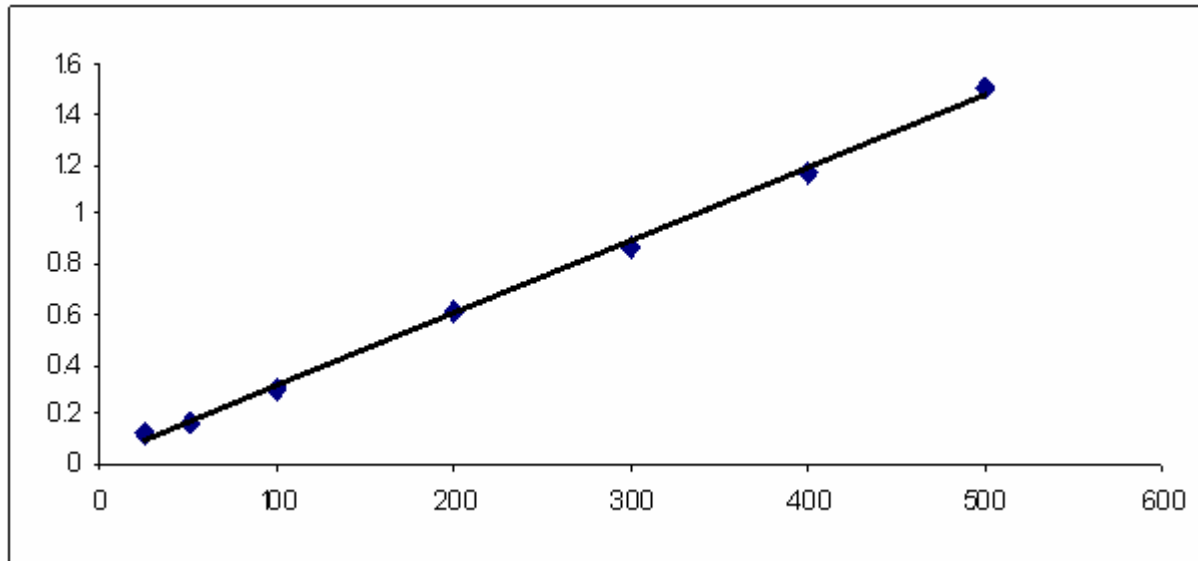
Conclusions



Linearity:

The calibration curve is linear in the range from:

25 - 500 ng/ml



Regression coefficient $r = 0.998$ $y = 0.0029 X + 0.0215$

Sensitivity:

limit of detection (LOD) = $\frac{3.3 \times S.D}{S}$

LOD = **8 ng/ml**

limit of quantification (LOQ) = $\frac{10 \times S.D}{S}$

LOQ = **25 ng/ml**



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Precision

| % RSD | | | |
|-----------|-------------|--------------|--------------|
| Intra day | | | |
| Run | 25 ng/ml | 200 ng/ml | 500 ng/ml |
| 1 | 0.1298 | 0.6342 | 1.4370 |
| 2 | 0.1219 | 0.5849 | 1.4645 |
| 3 | 0.1200 | 0.6037 | 1.5054 |
| 4 | 0.0843 | 0.6199 | 1.4890 |
| 5 | 0.0890 | 0.6273 | 1.5475 |
| S.D | 0.0208 | 0.0198 | 0.1394 |
| RSD | 0.0833 | 0.0095 | 0.0279 |

| % RSD | | | |
|---------------|-------------|--------------|--------------|
| Inter days | | | |
| Days | 25 ng/ml | 200 ng/ml | 500 ng/ml |
| 1 | 0.0358 | 0.0032 | 0.0111 |
| 2 | 0.0833 | 0.0096 | 0.0279 |
| 3 | 0.1468 | 0.0073 | 0.0303 |
| 4 | 0.2030 | 0.0098 | 0.0076 |
| 5 | 0.0165 | 0.0122 | 0.0346 |
| Mean (RSD) | 0.0968 | 0.0079 | 0.0223 |

Accuracy

| Actual concentration (ng/ml) | Observed concentration. (ng/ml) | % Accuracy |
|-------------------------------------|--|-------------------|
| 25 | 24.965 | 99.86 |
| 200 | 203.204 | 98.40 |
| 500 | 499.344 | 99.82 |



Stability :

| Actual terbutaline Concentration (ηg/ml) | Stored at room temperature for 24hr | Stored at Refrigerator 2 -8°C for 15 days |
|---|--|--|
| | Recovery % | Recovery % |
| Low = 25 | 98.8 | 98.21 |
| Medium=200 | 99.7 | 99.04 |
| High = 500 | 100.23 | 99.37 |



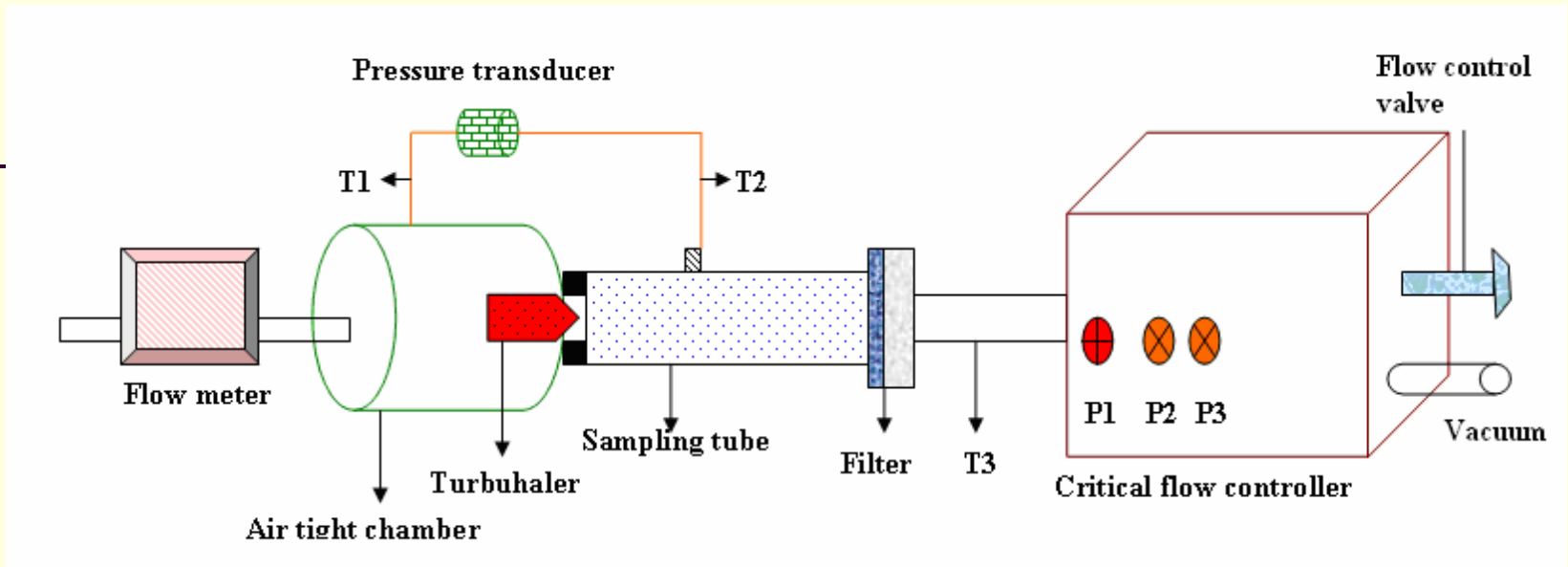
Application of The Method

This method can be used for :

Quality control parameters

- **Dose uniformity of inhaled product:** assessing the uniformity and accuracy of the dose delivered from Bricanyl Turbohaler
- **Andersen cascade impactors:** Measure the fine particle dose to examine the performance of inhaler

- Dose content uniformity



Instrument set up for Dose emission (Adapted from PhD thesis Nadarassan 2007)

| Dose number | Terbutaline |
|----------------|-------------|
| 2 | 65.2 |
| 3 | 47.8 |
| 4 | 63.6 |
| 54 | 92.2 |
| 55 | 76.7 |
| 56 | 64.7 |
| 116 | 67.0 |
| 117 | 73.5 |
| 118 | 84.5 |
| 119 | 82.1 |
| Average | 71.7 |
| S.D | 12.8 |
| RSD | 17.8 |

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Conclusions:

- The method described in this study is reliable, precise, and accurate.
- Additionally, MELC offered faster analysis times compared with conventional HPLC modes.
- The method was applied to determination of the content of the emitted dose of the Bricanyl Turbohaler.



THANK YOU

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