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Synthesis of Molecularly Imprinted Polymer for Selective Solid-Phase Extraction of Salbutamol from Urine Samples

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A highly selective methacrylic based molecularly imprinted polymer (MIP) was synthesized and applied for the separation and the pre-concentration of salbutamol in urine samples. Spectrophotometric determination of salbutamol was achieved using 2,6-dichloroquinone chlorimide as colorimetric reagent. The detection limit of the method was *ca.* 13 ng mL^{-1} in urine after pre-concentration of the samples by MIP-SPE and analysis with an optimized and sensitive spectrophotometric method. The linear dynamic range for salbutamol determination in urine was 0.04-0.75 μg mL^{-1}. The recovery for the affinity based solid-phase extraction (SPE) with the MIP was more than 96 %.

Key Words: Salbutamol, 2,6-Dichloroquinone chlorimide, Molecularly imprinted polymer, Solid-phase extraction.

INTRODUCTION

Salbutamol [2-(*tert*-butylamino)-1-(4-hydroxy-3-(hydroxyl ethyl)phenyl)ethanol], also known as albuterol, is a β-adrenergic receptor agonist, which is used as a bronchodilatator in the treatment of reversible bronchospasm[1]. The most commonly used methods for the analysis of salbutamol are gas and liquid chromatographic methods, flow injection analysis, chemiluminescence, electrophoresis, thin layer chromatography, normal and derivative spectrophotometric methods. Furthermore the use of spectrophotometric detection for salbutamol based on the formation of colour complexes has also been reported[2-7]. The methods have been reported are non-selective and are affected by the presence of compounds that contain redox reactive functional groups.

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Molecular imprinting has been recognized as a promising technique for the development of molecular recognition-based separations and sensing systems, where the molecule to be recognized is added to a reaction mixture consisting of a cross-linking agent, a solvent and a functional monomer that possesses functional groups capable of interacting with the target molecule. Binding sites in the resultant polymer include functional groups that originate from the functional monomer and which can be constructed according to the shape and chemical properties of the target molecule. Following removal of the target molecule the molecularly imprinted complementary binding sites exhibit a high degree of selectivity and affinity for the template molecule[8]. A high degree of selectivity is particularly desirable when extraction of a single compound from a complex matrix is required. Consequently the use of molecularly imprinted polymers (MIPs) in such analyses is an attractive option, particularly when used as molecularly selective sorbents in solid-phase extraction (SPE)[9]. The aim of the present research was to study the affinity-based separation and pre-concentration of salbutamol in urine samples using MIP-SPE with spectrophotometric detection based on the reaction of 2,6-dichloroquinone chlorimide (DCQ) (an electron acceptor) with salbutamol[10] (an electron donor).

EXPERIMENTAL

A Shimadzu Model 160A UV-Visible double beam spectrophotometer with quartz cells of 1.0 cm was used for the analysis of all samples. A pH meter was used for the pH checking of the universal and the ammonia buffer solutions. Digital scales for precise weighting, soxhlet apparatus, laboratory mortar, laboratory pestle and water bath for the MIP synthesis were also used.

All chemicals and solvents used were at least of analytical or pharmaceutical grade. All solutions were prepared in double distilled water. 2,6-Dichloroquinone chlorimide (DCQ) was supplied by the Aldrich Company (Natick, MA, USA). Fresh solutions of DCQ (0.1 % w/v) in isopropanol, universal and ammonia buffer solutions of pH 9.0 were freshly prepared immediately prior to use. Standard solution of salbutamol (2.0 mg mL^{-1}) was prepared in water. All solvents and the methacrylic acid were purchased from the Merck Company (Darmstadt, Germany). Ethylene glycol dimethacrylate and azo-N,N'-diisobutyronitrile (AIBN) were obtained from the Fluka (Buchs, Switzerland).

Molecularly imprinted polymer (MIP) preparation: To manufacture the salbutamol imprinted polymer AIBN (initiator 0.1 mg) and 0.5 mmol of salbutamol were dissolved in 5 mL of chloroform in a 20 mL glass tube. Methacrylic acid (MAA, 1.7 mmol) and ethylene glycolmethacrylate (EDMA, 10.5 mmol) were added to this solution and the mixture was degassed using nitrogen sparging for 10 min. The tube was sealed and heated in water bath at 50 ºC for 30 h. At the conclusion of the heating process a dense rigid polymer network that was white in colour had been produced. The tube was destroyed and the monolithic polymer that remained was ground using a mortar and pestle. In order to remove the residual monomers

and salbutamol the polymer was subjected to extraction using a Soxhlet extraction apparatus and a mixture of methanol-acetic acid in an 8:1 v/v ratio. In order to ascertain whether the extraction process was effective the concentration of salbutamol in the extraction solvents was determined using colorimetric method based on the charge transfer complex formation that occurred between salbutamol and the DCQ reagent. The polymer was ground once again using a mortar and pestle to effect particle size reduction and the resultant material was sieved through a 40 μm sieve with the aid of distilled water. The resultant fine particles were removed by decantation in acetonitrile and used to evaluate their potential for extraction of urine samples. A control, non imprinted polymer (NIP) was also manufactured in a similar manner by polymerization without salbutamol and the control polymer was used to establish the existence of nonspecific binding sites for salbutamol.

RESULTS AND DISCUSSION

Spectrophotometric determination of salbutamol: A previously reported procedure[10] was used as the method of choice for the determination of salbutamol in the presence of the DCQ reagent. Since DCQ is an electron acceptor and the benzene ring in salbutamol and isoxsuprine molecules consist of the electron rich groups, a $\pi \rightarrow \pi^*$ CT complex can be formed. The chemical structure of salbutamol and isoxsuprine in addition to that of the charge transfer complex that is formed between salbutamol and DCQ are depicted in Fig. 1.

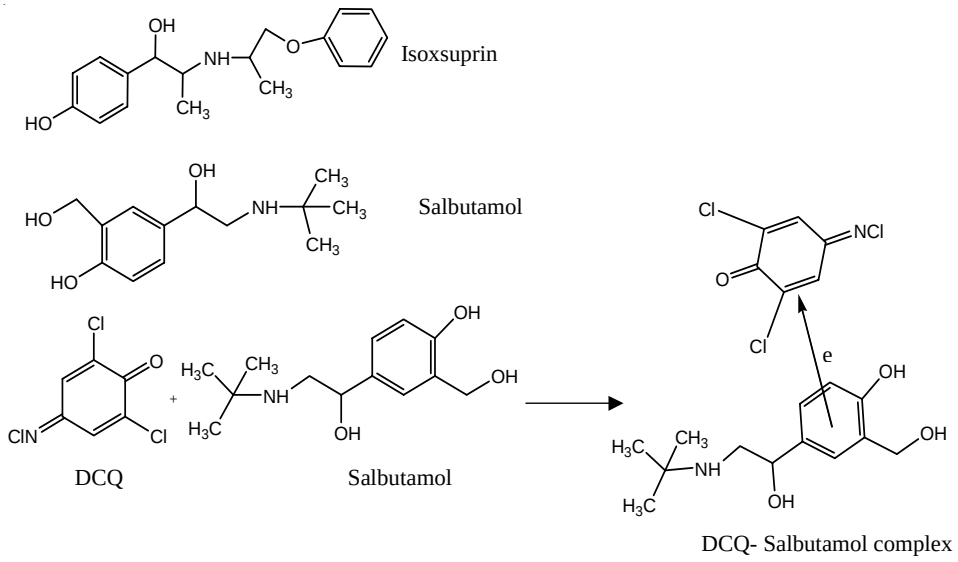

Fig. 1. Chemical structure of isoxsuprine and salbutamol molecules and the charge transfer complex formation reaction between the DCQ and the salbutamol

MIP evaluation using colorimetry: The molecular recognition ability of the MIP was evaluated using colorimetry. A fixed amount of MIP powder was added to a fixed volume of aqueous sample, containing 2 μg mL^{-1} of salbutamol. After 1 h, the target molecule had been adsorbed was removed from the MIP using methanol. This procedure was also performed with the NIP. The results of this evaluation are depicted in Fig. 2 and reveal that the salbutamol reaction with the DCQ following extraction from an aqueous solution with MIP and that of the NIP, respectively. It is clearly evident that the capability of the MIP to adsorb salbutamol is substantially higher than that of the NIP thereby providing evidence for the existence of a selective site for salbutamol sequestration on the MIP as compared to that of the NIP. In another evaluation of the selectivity of MIP for salbutamol the adsorption of isoxsuprine, as structurally similar molecule to salbutamol was investigated. As can be seen in Fig. 3 there is minimal difference for the adsorption of isoxsuprine between the MIP and NIP materials used. Therefore the selective sites for salbutamol sequestration that are present on the MIP, do not appear to demonstrate as high an affinity for the uptake of isoxsuprine as for salbutamol. Furthermore, it can be deduced that mechanism of adsorption of isoxsuprine are similar for both the MIP and NIP polymeric materials.

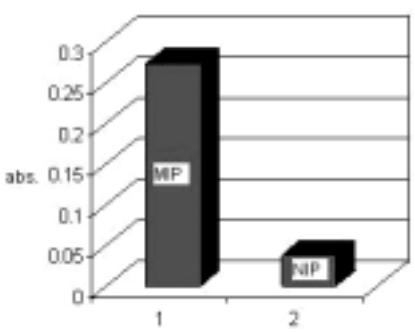

Fig. 2. Comparison of salbutamol adsorption using the MIP and NIP polymers. The experimental conditions used for the extraction of salbutamol from aqueous solution were 100 mg MIP and 50 mL salbutamol (0.6 μg mL^{-1}) for 1 h

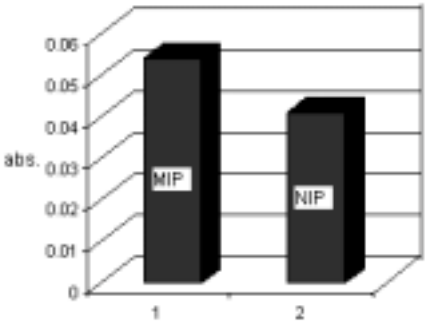

Fig. 3. Comparison of isoxsuprine adsorption using the MIP and NIP polymers. The experimental conditions used for the extraction of isoxsuprine from aqueous solution were 100 mg MIP and 50 mL isoxsuprine (0.6 μg mL^{-1}) for 1 h

Calibration curve and detection limit using the MIP-SPE: The calibration for the MIP-SPE determination of salbutamol was carried out under the specified optimal conditions. The calibration graph is characterized by a linear range from 0.04 to 0.75 μg mL^{-1} and an equation described by A = 0.734 C (μg mL^{-1}) + 0.111 with correlation coefficient equal to 0.9988. The detection limit of this method was calculated about 13 ng mL^{-1}.

Interference studies: All analyses, in which the reactivity of the salbutamol phenolic group is essential to generate a response, will be negatively affected by the presence of other compounds that are readily oxidized. Therefore the use of the MIP-SPE technique provides an opportunity to overcome this challenge as salbutamol is preferentially adsorbed whereas other similar materials will not be. The use of the proposed MIP-SPE method was applied to the determination of salbutamol in urine samples without any additional analytical artifacts or challenges. In addition, the presence of compounds such as starch, lactose, glucose, ascorbic acid, urea and uric acid in concentrations up to 100 times higher than that of salbutamol did not result in any interference in analysis of urine using this method.

Analytical application: The proposed method has been successfully used for the determination of salbutamol in spiked human urine samples. In order to evaluate the potential of this method, an aliquot of aqueous salbutamol standard solution was added to urine and diluted to between 5 to 20 times to reach a total sample volume of 150 mL and a salbutamol concentration range of between 0.04-0.75 μg mL^{-1}. 0.1 g of MIP was added to the urine and the container was agitated for 0.5 h. The sample was filtered through filter paper and washed with 4 mL of dichloromethane and then recovered with 3 mL methanol. Following extraction the sample was transferred to a 5 mL volumetric flask and evaporation of the methanol achieved using nitrogen 1 mL of buffer, prepared as previously described and 1 mL of the DCQ reagent solutions were added and to achieve a total volume to 5 mL. The colour was allowed to develop over 15 min and the resultant absorbance was measured at a wavelength of 620 nm. A calibration curve was constructed and the salbutamol concentration was calculated. Table-1 lists a summary of the salbutamol concentrations in spiked urine samples.

TABLE-1
CONCENTRATION OF SALBUTAMOL IN SPIKED URINE

Salbutamol	Added ($ng\ mL^{-1}$)	Found* ($ng\ mL^{-1}$)	Recovery (%)	RSD (%)
Urine diluted 5 times	80	82.5	103.1	4.5
	100	102.6	102.6	2.1
	50	48.7	97.4	3.8
Urine diluted 20 times	50	48.2	96.4	3.2
	100	97.8	97.8	2.9

*Mean of 6 measurements.

Conclusion

In the present study a molecular imprinted polymer was prepared and used for the determination of salbutamol in human urine. The evaluation of the MIP/salbutamol reaction by colorimetric determination was shown to have a degree of selectivity of due to the affinity of the novel sorbent for salbutamol. It has been proven that by combining affinity based MIP-SPE and a simple spectrophotometric method a precise, selective and sensitive determination of salbutamol in low concentration is possible.

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REFERENCES

1. R. Berges, J. Segura, X. De La Torre and R. Ventura, *J. Chromatogr. B: Biomed. Appl.*, **723**, 173 (1999).
2. K.K. Bhatt, S.A. Shah and S.S. Pandya, *Indian Drugs*, **36**, 524 (1999).
3. K.P.R. Chowdary and R.G. Devala, *Indian Drugs*, **34**, 283 (1997).
4. R.S. Bakry, O.A. Razak, A.F.M. El Walily and S.F. Belal, *J. Pharm. Biomed. Anal.*, **14**, 357 (1996).
5. T.D. Burns, N. Chimpalee, D. Chimpalee and K. Leiwongcharoen, *Anal. Chim. Acta*, **260**, 65 (1992).
6. N.V. Naidu, D.V. Naidu, C.V. Rajeswari and P.R. Naidu, *Acta Chim. Hung.*, **126**, 821 (1989).
7. N. Geeta and T.R. Baggi, *Microchem. J.*, **39**, 137 (1989).
8. T. Takeuchi and J. Haginaka, *J. Chromatogr. B*, **728**, 1 (1999).
9. A. Beltran, E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington and F. Borrull, *Anal. Chim. Acta*, **597**, 6 (2007).
10. G.G. Mohamed, S.M. Khalil, M.A. Zayed and M. Abd El-Hamid El-Shall, *J. Pharm. Biomed. Anal.*, **28**, 1127 (2002).

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