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Review Article

Human Saliva: It's Utility as a Biological Matrix in the Quantification of Pharmacological Active Agents (Drugs)

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ABSTRACT

Quantification of pharmacological active agents (drugs) in biological fluids is very often performed to evaluate patient adherence and track the effectiveness of the dose that was administered. Though mostly used in diagnostic and forensic/toxicology analyses, a number of reports have utilized saliva as an alternate matrix to whole blood, plasma, serum and urine in drug concentrations determinations. The objective of the study was to present summary of numerous studies on saliva as a biological matrix.

Prediction of Saliva-Plasma transfer ratios by mathematical models combined with pharmacokinetic, and physiological data supporting interpretation of saliva drug concentrations, have greatly enhanced the value of saliva as a biological matrix for drug concentrations quantification.

Keywords: Human saliva; Biological matrix; Quantification of drugs

INTRODUCTION

The quantification of pharmacological active agents in clinical samples of patients administered with these active compounds is necessary to understand their pharmacodynamics and pharmacokinetics. Tissue or biological fluids (namely whole blood, plasma, serum, and urine) are usually the clinical matrices of interest. However, other biological fluids such as cerebrospinal fluid, saliva, sweat, synovial fluid, tears are at intervals utilized [1,2].

Saliva is a thick colorless, opalescent extracellular fluid produced by salivary glands (parotid, submandibular, and the sublingual glands) and secreted in the mouth by salivary ducts [3]. It is composed of water (99%), mucus, protein (1%), mineral salts and contains important enzymes such as amylase, lipase, and lysozyme [4,5]. The fluid as it circulates in the mouth cavity collects bacteria cells, food debris, and white blood cells. Salivary composition and flow can be affected by many factors, including oral diseases [6,7]. The flow rate of un-stimulated saliva is approximately 0.3 ml/min-0.4 ml/min while its daily production is between 0.5 and 1.5 liters. Like blood, the pH of healthy saliva is neutral or slightly alkaline (approximately pH 7.4).

Saliva does have the following functions: (i) makes swallowing food easier, (ii) washes away food debris (cleaning effect), (iii) fights off bacteria entering the mouth (antibacterial effect) as it contains antimicrobial agents such as hydrogen peroxide, lactoferrin, and lysozymes, (iv) prevents caries (pH buffering effect), (v) promotes remineralization of teeth, (vi) protects mucous membrane (lubricating effect) [8,9].

Disruptions in saliva secretion probably by stones (sialoliths) in the gland ducts, HIV-AIDS and autoimmune disorders, as well as infections or tumors in the salivary glands could lead to oral conditions such as gum disease, oral candidiasis, tooth decay (caries), as well as respiratory tract infections [10]. Due to the low active substance concentrations present in the saliva, sensitive and specific methods are employed [11]. Numerous analytical techniques have been used to monitor administered pharmacological active substances concentrations in saliva. Examples of such analytical techniques include electrochemical, immunological, chromatographic, and spectroscopic techniques.

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Chromatographic methods such as high performance liquid chromatography or gas chromatography is mostly used either as hyphenated or non-hyphenated system. Hyphenation is an on-line combination of a chromatographic system and one or more spectroscopic detection techniques.

Although literature review has resulted in the identification of numerous references regarding the use of saliva as a biological matrix, the present study, attempted to provide summary of studies on saliva as a biological matrix, with emphasis on assay methodology; factors accounting for observed variability in its use; and various analytical techniques utilized.

LITERATURE REVIEW

Clinical analysts do occasionally prefer to utilize saliva as a biological fluid of interest because of a number of merits associated with the biological matrix [12-15]. Such merits include:

1. Non-invasive sampling technique.
2. Less discomfort to workers participating in drug monitoring.
3. Straight forward sample collection. For instance, patient can take the sample at home and deliver it to the laboratory.
4. Less complex sample storage and transport arrangements than those for blood.
5. Ethical approval for sampling is more easily obtained.
6. Valuable diagnostic material where good correlation between the concentration of the test drug in the blood and saliva exist.
7. Handy matrix for therapeutic drug monitoring particularly in elderly, in whom blood collection is hindered by the fragility of veins.
8. Rapid procedure that may be applied to different patient groups regardless of age.
9. Relatively free of blood constituents, therefore can be easily prepared for testing by conventional drug screening and confirmation methods.

However, demerits associated with saliva limit clinical analysts utilizing it as biological matrix in therapeutic drug monitoring [16,17].

Such demerits include:

1. Sensitivity is lowered by contamination. Contaminants may include food debris, bacteria, epithelial cells; and hand-to mouth behavior prior to sample collection.
2. Effective as a surrogate for blood only in highly-exposed populations (heavy metals and organometallic drugs).
3. Requires a qualified phlebotomist.
4. Composition of saliva is different from that of blood and therefore might call for development of new, or modification of existing, extraction procedures.
5. Often, the concentrations of the monitored drugs in saliva are lower than in blood.
6. Standardized testing procedures requirements.
7. Changes in pH.
8. Very low compliance-relevant concentrations for a number of drugs.

Sample collection: A number of different procedures have been applied in collecting saliva samples of which some involve stimulating saliva production, while others target collection of unstimulated saliva. Unstimulated saliva can be collected by the draining method, and is done by allowing saliva to drip from the mouth into a collection vial [18]. The simplest technique to collect stimulated saliva involves cheek, lip or tongue, movements without the use of an external stimulus [19,20]. Others include (a) mechanical methods of stimulating saliva production namely chewing paraffin wax, gum base, rubber bands, Teflon, (b) placing a lemon drop or citric acid in the mouth to provide a gustatory stimulus for saliva production, (c) combining stimulation and collection of the saliva using absorbent materials such as cotton balls or cotton rolls. The saturated absorbent material with saliva, is removed from the mouth and the saliva is extracted by centrifugation or by applying pressure to the material [18-24]. In general, disadvantages of stimulated spitting make non-stimulated spitting the most effective technique (because it gave the highest levels of drug concentration). Such disadvantages include: (i) absorption of some drugs by paraffin and, therefore, give erroneous results when saliva is tested for drugs or drug metabolites [25], (ii) paraffin may contain compounds that affect chromatographic analyses, thus leading to drug testing inaccuracy [25], (iii) salivary composition could be changed by some salivary stimulants and, therefore, affect the saliva-drug concentration. For example, the change in saliva pH by citric acid thus altering drug concentrations in the saliva, (iv) alteration of immunoassay drug test results by citric acid and cotton [19,26].

Sample preparation: Different procedures have been used for sample purification, namely

(a) cloude-point extraction [27], (b) deproteinization [28,29], (c) liquid–liquid extraction [30,31], and (d) solid phase extraction [32,33].

Sample storage: Centrifuged samples are often stored at −20 ◦C or −80 ◦C until required for analysis [16,34].

Stability of analyte(s) in sample: The stability of the analyte(s) in saliva samples may be assessed under different conditions namely

(i) six months of freezing at −21 °C or − 80 °C, (ii) three cycles of freezing (at least 12 hrs at −21°C or −80°C) and thawing at room temperature (20°C) before analysis, (iii) storage at 4°C or 8°C, re-analyzed after 4 h, (iv) at room temperature, re-analyzed after 2 h, (v) stored in HPLC auto sampler at room temperature, re-analyzed after 2 h or at 15°C for 72 h [16].

Studies have shown that selectivity (indicated by absence of interference resulting from the presence of endogenous compounds) as well as no carry-over effect has been observed in analyses performed using saliva as a biological matrix [16,35].

Active agent(s) determinations by various analytical techniques

Saliva has been utilized as a biological matrix in a number of therapeutic drug monitoring determinations or assessment of occupational exposure of health care personnel working with these active agents. Such quantifications and analytical techniques utilized include:

1. Inorganic elements (heavy metals)-spectroscopic methods (inductively coupled plasma mass spectrometry (ICP-MS)-[14,36,37];
2. Antiepileptic drugs-immunoassay methods (fluorescence polarization immunoassay FPIA, radioimmunoassay); chromatographic methods [38-40].
3. Antiretroviral drugs-chromatographic methods [41-43].
4. Anti-tubercular agents (linezolid, and moxifloxacin)-chromatographic methods [44].
5. Mycophenolic acid (indicated for autoimmune diseases, pediatric nephrotic syndrome, and acute rejection prophylaxis after solid organ transplantation)-chromatographic methods [36,45,46].
6. Analgesics-chromatographic methods [47-49].
7. Central nervous stimulants-chromatographic methods [50-54].
8. Sedatives-chromatographic methods [55-57].

In general, the use of saliva as an alternative biological matrix for whole blood, plasma or serum, urine has conflicting literature reports. For examples:

1. Saliva may not be a good substitute in the determination of heavy metal ions, except at higher levels of exposure [14],
2. Dolutegravir concentration in saliva was reported to reflect the pharmacologically active dolutegravir concentration in plasma [35],
3. Therapeutic drug monitoring using saliva as matrix is a suitable alternative for serum therapeutic drug monitoring of linezolid, but not for moxifloxacin due to a high variability in saliva-plasma ratios [45],
4. Thin Layer Chromatographic (TLC) technique for monitoring nevirapine in saliva was unsuccessful, however using HPLC technique, nevirapine concentrations in saliva and plasma could be determined [43], furthermore Vapaatalo et al. (1984) reported Thin-Layer Chromatography (TLC) methods to be unsuitable for use in the detection of amphetamines in saliva, due to low concentrations of amphetamines found in saliva [58],
5. Successfully used to monitor the concentration of carbamazepine and metabolite in patients' saliva [16],
6. Saliva concentrations reflect the pharmacologically active form of plasma mycophenolic acid (unbound drug levels), hence offer a more acceptable alternative biological fluid for drug concentration measurement [59,60],
7. Saliva cocaine concentrations paralleled those detected in the plasma [61], although study has reported that saliva cocaine concentrations may differ between stimulated and unstimulated collections [62]. Literature has shown that there appears to be a reasonable correlation between saliva and plasma cocaine concentrations as well as physiological effects [63,64],
8. Mathematical models that predict Saliva to Plasma (S/P) drug concentration ratios for acidic and basic drugs have shown good correlations[65],

For acidic drugs, $S/P=\frac{1+10(pHs-pKa) \times fp}{1+10(pHp-pKa) \times fs}$

While for basic drugs, $S/P=\frac{1+10(pKa-pHs) \times fp}{1+10(pKa-pHp) \times fs}$

Where, S=concentration of drug in saliva, P=concentration of drug in plasma, pKa=pKa of drug, pHs=pH of saliva, pHp=pH of plasma, fp=free (unbound) fraction of drug in plasma, fs=free (unbound) fraction of drug in saliva. The application of these equations assumes that, plasma pH is a constant at 7.4, fs has a value of 1 and drug protein binding is to be negligible in the saliva [22]. Theoretically using any of the equations, the plasma concentration of a drug given its saliva drug concentration can be predicted, if the pH is measured at the time of sample collection [19]. The normal pH of saliva varies from 6 to 8 [65]. It is also vital to note that the binding of drugs to plasma proteins varies from drug to drug, but may remain fairly consistent between individuals. Therefore, if S/P ratios can be shown to be predictable using mathematical models, and the databases on plasma pharmacokinetic, physiological, and behavioral data could be used to support interpretation of saliva drug concentrations, then this would greatly enhance the value of saliva as a matrix for drug testing.

CONCLUSION

Saliva, a thick colorless, opalescent extracellular fluid provides to human body buffering, cleaning, antimicrobial, lubricating effects. Dysfunction in saliva secretion could lead to disorders namely gum disease, oral candidiasis, tooth decay, as well as respiratory tract infections. Reports have described saliva as a biological matrix of interest in the quantification of active agents in body fluids. However, due to lack of good correlation with other alternative biological matrices (whole blood, plasma or serum and urine) clinical analysts do occasionally have reservation using saliva as a biological matrix. Such reservations are as a result of sample contamination, potential extraction procedures error, pH changes and other disadvantages associated with saliva as biological matrix.

A number of factors need to be considered prior to selecting saliva as a biological matrix namely: (i) route of administration, (ii) steady state drug concentrations, (iii) drug structural and physiochemical properties, (iv) purification procedure, (v) saliva-to-plasma transfer ratio (S/P), (vi) technique used to collect saliva as it affects the drug concentration, (vii) how concentrations of drug in saliva correlate with drug concentrations in other body fluids, and (viii) sensitivity of analytical technique to be employed.

Finally, if mathematical models can predict S/P ratios, and databases on plasma behavioral, pharmacokinetic, and physiological data supporting interpretation of saliva drug

concentrations, then the value of saliva as a biological matrix for drug quantification would greatly enhanced.

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