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Research Article

# ANALYTICAL AND BIOANALYTICAL PERSPECTIVES ON DOXAZOSIN FOR BIOSCIENCES AND THERAPEUTIC RESEARCH

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## ABSTRACT

Doxazosin, a selective antagonist of alpha-1 adrenergic receptor which is commonly applied for curing hypertension and benign prostatic hyperplasia. For pharmaceutical quality control and efficient treatment monitoring, doxazosin estimation in biological fluids must be done precisely. Numerous analytical and bioanalytical techniques developed for its detection are integrated in this comprehensive study, including spectrophotometric techniques, electrophoretic approaches, and chromatographic techniques (HPLC, UPLC, and LC-MS/MS). In the current study overview of doxazocin and its pharmaceutical significance along with physicochemical properties, analytical and bioanalytical methods, recent developments that improve analytical performance, bioanalytical method validation, various validation parameters are highlighted. This review provides a comparative evaluation of different methods, emphasising their benefits, drawbacks, and suitability for use in various academic, professional and industrial contexts.

**Keywords:** Doxazosin, Benign prostatic hyperplasia, Hypertension, Spectrophotometric techniques, Evaluation.

## INTRODUCTION

Doxazosin mesylate exhibits therapeutic efficacy in the treatment of benign prostatic hyperplasia and hypertension (Shrivastava, A *et.al.,* 2014). Because doxazosin specifically inhibits alpha-1 adrenoreceptor blockers, these medications are preferred for the treatment of benign prostatic hyperplasia (Shrivastava, A *et.al.,* 2014). The effects of doxazosin mesylate are the same as those of prazosin, although they last longer. It is used to treat benign prostatic hyperplasia and hypertension in order to alleviate the symptoms of urinary blockage. Doxazosin is a selective alpha one adrenoreceptor blocker that was first created as an antihypertensive (Shrivastava, A *et.al.,* 2014). Doxazosin is a selective antagonist of alpha one adrenoreceptor that was first created as an antihypertensive medication. First administered at a dose of 1 mg/d, doxazosin should be titrated over 1-2 weeks to a maximum dose of 8 mg/d. Titration lowers the chance of cardiovascular adverse effects from the first dose (Shrivastava, A *et.al.,* 2014). Doxazosin and the comparator medication have been shown to be equally effective in treating mild to moderate hypertension in the majority of comparative trials. Elderly people, Black people, smokers, and people with coexisting medical conditions like renal failure, hypercholesterolemia, non-insulin-dependent diabetic mellitus (NIDDM), and respiratory disorders have all been treated with it. In individuals whose hypertension cannot be treated with monotherapy, doxazosin has also been used effectively in conjunction with angiotensin-converting enzyme inhibitors, diuretics, calcium channel antagonists, and β-adrenoceptor antagonists. When it comes to treating urinary symptoms linked to benign prostatic hyperplasia, doxazosin seems to be a promising medication. Doxazosin treatment improves several objective and symptomatic parameters and raises peak and mean urine flow rates, just as other α1-adrenoceptor antagonists (Fulton, B *et.al.,* 1995).

## MATERIALS AND METHODS

### Physicochemical properties of doxazocin

Doxazosin mesylate is a 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-ylcarbonyl) piperazine

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methane sulfonate, is a well-known and efficient quinazoline derivative that specifically blocks the α-1 adrenoreceptor to treat benign prostatic hyperplasia and hypertension (Sreevatsav SK *et.al.,* 2013).

**Drug profile**

**Doxazosin**

Doxazosin is a fast-acting, oral, selective factor Xa inhibitor that belongs to the Novel Oral Anticoagulants (NOCS) class of medications. Edoxaban inhibits the coagulation cascade's essential protein, factor Xa, which stops the progressive amplification of the protein factors required for blood clotting.

**IUPAC name**

2-[4-(2,3-dihydro-1,4-benzodioxine-2-carbonyl) piperazin-1-yl]-6,7 dimethoxyquinazolin-4-amine**.**

**Molecular formula and molecular weight**

$C_{23}H_{25}N_5O_5$ ; 451.47g/mol. Solubility and physical state: it is a white to off white solid which is soluble in water and acetonitrile. Category: Oral anti-coagulant drug. Brand name: Cardura, Carduraxl. Melting point and Density: 276ºC to 278ºC; 1.3-1.5g/$cm^3$. Storage conditions: 25 $^0$C Dosage form and Strength: For tablets 2mg, 4mg, 8 mg.

**Pharmacodynamics**

When doxazosin is given to patients, clotting time tests such the international normalized ratio (INR), prothrombin time (PT), and activated partial thromboplastin time (PTT) are enhanced.

**Pharmacokinetics**

**Absorption**

Peak plasma doxazosin concentrations are seen 1-2 hours after oral dosing. 62% is the absolute bioavailability.

**Volume of distribution**

Doxazosin has a volume of distribution of 1.0–1.9 L/kg (Elliott HL *et.al.,*1987, Cubeddu LX *et.al.,* 1987). volume of distribution of doxazosin in steady state is 107L.

**Route of elimination**

Doxazosin is eliminated primarily as unchanged drug in urine.

**Protein binding and Half –life**

Doxazosin's plasma protein binding is thought to be 98%and 10 to 14 hrs half-life (Kaye B *et.al.,*1986).

**Toxicity**

over dosage may leads to fluctuations in heart rate, reduction of blood pressure and also symptoms like drowsiness. Removal of excessive doxazosin which remained unabsorbed from the gastrointestinal tract, correct hypotension, and closely monitor vital signs (Satar S *et.al.,* 2005).

**Pharmacological significance**

PTSD-related nightmares, ureteral stones, hypertension, and benign prostatic hyperplasia are all treated with the drug doxazosin (Kim J *et.al.,*2023).

**FDA approved Indications** (Kim J *et.al.,*2023)

Benign Prostatic Hyperplasia, Hypertension (immediate release only)

**Non-FDA Approved Indications** (Kim J *et.al.,*2023)

Ureteral Stones, PTSD Associated Nightmares

**MOA**

Doxazosin is a quinazoline derivative that functions at the post-synaptic receptor as a competitive alpha1-antagonist (Kim J *et.al.,*2023).

**In Hypertension**

vasodilation of arterioles and veins caused due to the inhibition of post-synaptic alpha1-adrenergic receptors resulting in decreased total peripheral resistance and blood pressure (Kim J *et.al.,*2023).

**In Benign Prostatic Hyperplasia**

Inhibiting alpha1 receptors resulting the decrease in resistance of urethra and enhancing the flow of urine (Kim J *et.al.,*2023).

**Analytical methods for doxazocin estimation**

Various analytical techniques are existed in analytical method development to estimation of doxazocin. The most common methods that are used are listed as: Spectroscopic methods, Spectrofluorimetric method, UV-Visible spectrophotometry, Derivative spectroscopy, IR Spectroscopic method, NMR Spectroscopic method, Mass Spectroscopic method: Chromatography method HPLC, UPLC, HILIC, HPTLC, Partition chromatography, Adsorption chromatography, Ion exchange chromatography. Electrochemical methods: Voltammetry, Polarography, Conductometry.

**Spectroscopic methods**

The most common methods that are used from the ancient times for the analysis are spectroscopic methods. The detection and evaluation of substances using spectrophotometric approaches depend on an association between a compound's molecular structure and the properties of its absorption bands, including their location and strength, in the electromagnetic spectrum (Marczenko Z *et.al.,*2000). Because of its distinct fluorescence properties, spectrofluorimetric as an analytical method offers a clear identification of the compounds included in the sample. Nanogram levels of analysis are possible for the substances (Nahata A *et.al.,*2011). For spectrophotometry in the UV and VIS areas, the differentiation of atomic spectra offers significant benefits. It is the key to potentially improving the resolution of overlapping bands, detecting poorly absorbed peaks caused

by impurities or admixtures in solution or structural issues, enabling precise λmax determination of specific analyte species, and boosting spectrophotometric procedures sensitivity. Furthermore, it is a very good background removal method (Sommer L *et.al.,* 1989). Doxazosin could only be determined using one first derivative spectrophotometry method (Bebawy LI *et.al.,*2002). some spectroscopic methods used in analytical method development are mentioned in table 1.

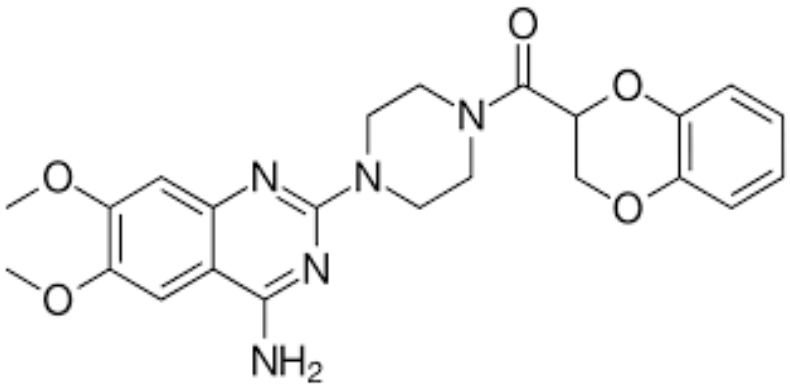


**Figure 1.** Chemical structure of Doxazosin.

**Table 1. Spectrometric Methods.**

| S. No. | Spectroscopic method | wavelength | Stoichiometric analysis | Application | Reference |
|---|---|---|---|---|---|
| 1. | Simple spectrophotometric assay | 330 nm | Linearity: 1.0×10−5 and 5.0×10−5 M LOD: 1 × 10- 5 M | Comparison with voltametric method | (Altiokka G *et.al.,*2001) |
| 2. | First derivative spectrophotometric method | 256 nm | Linearity: 8–120 µg/ml | Bulk pharmaceuticals and tablets | (Bebawy L.I *et.al.,*2002) |
| 3. | fluorimetry methods | 336 nm, λex = 400 and λem = 475 nm | Linearity: 0.02-0.22µg/Ml LOD: 0.01 LOQ:0.02 | Pure and tablets | (Ayad M.M *et.al.,*2012) |
| 4. | fluorimetry methods | 547 nm, λex = 340 and λem = 570 nm | Linearity: 2 - 14 µg $mL^{-1}$ and 1-10 µg $mL^{-1}$ | Tablets | (Ammar R.A *et.al.,*2014) |
| 5. | methods based on acid-dye method | 418, 414, 425, and 426 nm | Linearity: 16, 1.0-12, 4.0-50 µg/ml | Pure and tablets | (El Sheikh *et.al.,*2012) |
| 6. | methods based on acid-dye method | 403 and 410 nm | Linearity: 3-18 µg/ml, 3-20 µg/ml | Tablets | (Aydogmus Z *et.al.,* 2009) |
| 7. | UV spectrophotometric method | 245 nm | Linearity: 2-14 µg/mL | Bulk materials and pharmaceutical dosage forms | (Priyanka S. Yadav *et.al.,*2022) |

**Chromatographic methods**

The late 1960s saw the development of HPLC, which was soon applied to pharmaceutical analysis. As the need for purity testing of pharmaceutical goods and bulk medicines grows, it has evolved into a widely applicable analytical technique that offers quick and flexible separation options (Shrivastava, A *et.al.,*2012). Although not a novel technology, HILIC has recently experienced a resurgence thanks to the development of reliable and repeatable stationary phases (Cubbon S *et.al.,*2010). HILIC can resolve an analyte and its counter-ion in the same analysis, separate analytes with widely different polarities in a

reasonable amount of time, and enhance retention of highly polar analytes, in addition to increasing sensitivity with electrospray LC-MS (Zhang W *et.al.,*2022). Doxazosin was quantified using the HILIC-MS/MS method, which was created and verified (i H.Y *et.al.,*2008). Post-extraction analyte spiking was used to evaluate matrix effects, which this approach was said to be free of.The combination of mass spectrometry and chromatography has garnered significant attention during the last forty years or more. However, because they do not have direct access to the equipment, most chromatographers are compelled to rely on service providers due to the complexity of mass spectrometers. Therefore, they are not able to react promptly to the results of the analysis, which makes the detector extremely challenging to operate. However, the HPLC when combined with mass spectrometry allows identification and quantitative measurement of compounds that cannot be fully analysed chromatographically.For the determination of doxazosin, two LC-MS techniques were also discovered (Chytil L *et.al.,*2010, Erceg M *et.al.,*2010). Both techniques can be used to biological matrices and offer a notable level of sensitivity.

Since its launch in early 2004, UPLC has been progressively incorporated into industrial labs, particularly in the pharmaceutical sector, because of its high resolution, rapid speed, and solvent savings. When compared to the HPLC method utilizing a traditional 3.5 µm column, a UPLC method is used that can reduce time upto 80% without compromising separation performence. Additionally, the UHPLC technique development scouting time is much decreased by a substantially shorter run duration (Chen *et.al.,*2013).When combined with automated sample application and densitometric scanning, the modern HPTLC approach is sensitive and completely reliable, making it suitable for use in both qualitative and quantitative analysis. HPTLC is a helpful tool for precise identification since it can create chromatographic fingerprints that can be viewed and stored as computer images (Srivastava *et.al.,*2011). HPTLC has several advantages over TLC, such as faster separation, higher resolution, and five to ten times more sensitive detection without requiring prior extraction (Andola Harish *et.al.,*2010). Visible chromatograms are produced using HPTLC, providing data about the sample in detail. Several samples analyzed simultaneously in order to compare reference and test samples for identification (Shepherd RW *et.al.,*1978). Various chromatographic analysis of doxazosin studies is mentioned in table 2.

**Table 2.** Chromatographic methods.

| Method | Chromatographic conditions | Mobile phase | Linearity range | Detection wavelength | Application | Reference |
|---|---|---|---|---|---|---|
| Reverse phase HPLC method | A stainless steel Chromolith RP-18 column measuring 100 x 4.6 x 10 µm has been utilized. | A 40:60 ratio of phosphate buffer to methanol makes up the mobile phase of an isocratic technique. | 50-150 µg/ml | 251nm | Tablets | (Sreevatsav SK *et.al.,*2013) |
| LC assay method | LiChroCART: Lichrosphere100 RP-18 column (five [micro]meters, 250 x 4.0 mm) | DXM degraded in acidic (1M HCl), alkaline(1M NaOH) and hydrogen peroxide conditions, | 1.0-300 [micro]g [mL] | | Tablets | (Rao, K.S., *et. al.,*2012) |
| HPLC-UV method | A 5 um column with an interior diameter of 250 x 4.6 mm is called X Terra ® RP18. | Use 0.03M potassium hydrogen phosphate buffer to get the pH down to 3.2 after adding orthophosphoric acid: Acetoni trile (60:40v/v). | 48-144 µg/ml | 245 nm | Bulk and Pharma ceutical Preparation | (Kulsum S *et.al.,*2011) |
| HPLC-UV Gradient | Kromasil C18 column with column measurements 250 × 4.6 mm, 5.0 µm | A: 0.05 ml of ACN-diethylamine, B: methanol, and C: 10 mM ammonium acetate (A:B:C) was: 60:40:0:0 for eight minutes, 60:20:20:0 for one minute, 60:0:40:0 for five minutes, and an additional 60:40:0:0 gradient for one minute for system equilibration. | 2-500 µg/ml | 254 nm | Tablets | (Shrivastava A *et.al.,*2012) |

| | | | | | | |
|---|---|---|---|---|---|---|
| HPLC-F method | ODS[Octadecylsily columns] hypersil column | At a flow rate of 1.0 ml/min, acetonitrile: 10 mM ammonium acetate (40:60) Are used | 1.0 50.0 ng/ml | . λex = 246 nm, λem = 376 nm | pharmacokinetic and bioequivalence study | (Wongsinsup C *et.al.,*2007) |
| HILIC MS/MS | 250 x 4 mm, 5 µm Atlantis HILIC Silica column. | ACN/ammonium formate mobile phase (100 mM, pH 4.5) (93:7 v/v) | 0.2–50 ng/ | mL ESI MS/MS | Pharmacokinetic study | (Zhang W *et.al.,*2012) |
| UPLC MS/MS | This 2.1 x 50 mm column (ACQUITY UPLC BEH C18 column, Waters) is packed with 1.7 µm particles and is made to endure 15,000 psi. | Pentadecafluorooctanoic acid in acetonitrile (0.05 (w/v)) and water (0.05 (w/v)) were the contents of mobile phases A and B, respectively. The gradient program looked like this: 0.4 ml min−1 for 0–1.45 minutes, 1 ml min−1 for 1.45–1.55 minutes, and 1 ml min−1 for 99%–10% mobile phase A, and 1.55–2 minutes for 10% mobile phase A. | 0.2 and 100 ng/ml | moni toring reactionof the transitions: m/z 452→344 | Human Plasma | (Erceg M *et.al.,* 2010) |
| HPTLC | Silica gel 60 F254 | A: 0.05 ml of ACN-diethylamine, B: methanol, and C: 10 mM ammonium acetate (A:B:C) was 60:40:0:0 for eight minutes, 60:20:20:0 for one minute, 60:0:40:0 for five minutes, and so on. | | 277 nm | Tablets | (Sane R.T *et.al.,* 2002) |

**Electro analytical methods**

In the pharmaceutical industry, as well as in the majority of analytical chemistry domains, conventional electrochemical techniques are now specific, deliberate, quick, and affordable. Among all trace pharmaceutically active chemical analyses, they are arguably the most adaptable (Shrivastava *et.al.,* 2012, Sharma J *et.al.,*2013). By using electroanalytical techniques, many pharmaceutically relevant problems can be readily resolved with an elevated level of analytical parameters like precision selectivity, accuracy, sensitivity in a way that is remarkably reproducible (Zuman P et.al., 2006). Techniques such as Differential pulse voltammetry (DPV) and square wave voltammetry (SWV) can be utilized to determine it based on its mechanism of oxidizing of the amine group and absorptive stripping of doxazosin. Doxazosin levels in urine and formulations were measured using both techniques. The measurement of doxazosin using SIAP-superimposed increasing amplitude pulse and SCAP-superimposed constant amplitude pulse polarographic techniques are presented. This technique relies on the quinazoline group present in the doxazosin molecule, which can be reduced with the mercury electrode that contains two electrons on it. (Altiokka G et.al.,1998).

**BIO ANALYTICAL METHOD VALIDATION**

Bioanalytical procedures are often used for the identification of concentration of a drug molecule and its metabolites amount in a biological matrix such as blood, urine, serum, saliva etc (Sapkal DB *et.al.,*2023). The biological matrix concentration can be determined using chromatographic methods such as HPLC, liquid chromatography–tandem mass spectrometry, etc. Biological analysis is essential for the research of evaluation of pharmacokinetic parameters, evaluation of pharmacodynamic activity and toxicological evaluation during development of a drug substance. In addition to drugs and metabolites, the bioanalytical method analyses both small and large molecules, including proteins, aminoacids and peptides. The bioanalytical method is essential in many other research areas, such as forensic analysis, doping control, and the creation of biomarkers for the investigation of many diseases. The validation of bioanalytical methods is necessary for the quantitative measurement of many different analytes in biological and physiological matrices, and this methodology is highly beneficial in both non-human and human clinical pharmacology (Sapkal DB *et.al.,*2023). It includes the sampling, sample preparation, analysis, calibration, data evaluation & reporting of the all activity related to bio-

analytical activity. In modern bio analysis good sample preparation & hyphenated instrumentation are required for better results with their accuracy. Bio-analysis plays an important role in drug development & essential for study of following during drug development & drug discovery (Kachave RN *et.al.,* 2020). To ascertain the amount of doxazosin mesylate present in human plasma, a straightforward, quick, and selective LC–MS technique utilizing a single quadrupole mass spectrometer was created and verified (Ning Ma *et.al.,*2007). For the simultaneous measurement of doxazosin and verapamil in human serum, a quick and accurate technique utilizing LC–MS/MS has been devised. For quantification, trimipramine-d3 was employed as an isotopic-labelled internal standard (Lukas Chytil *et.al.,*2010). Utilizing LC–MS/MS, an enantioselective and sensitive technique was created and verified for the identification of doxazosin enantiomers in human plasma. In an alkaline environment, ethyl ether/dichloromethane (3/2, v/v) was used to extract the enantiomers of doxazosin from plasma (Ke Liu *et.al.,* 2010).

**Table 3.** Bioanalytical studies data.

| Method | Chromatographic conditions | Mobile phase | Flow rate & retension time | Detection | Application | Reference |
|---|---|---|---|---|---|---|
| LC–MS method | A Thermo Hypersil-Hypurity C18 was used for the LC separation. | methanol, acetonitrile (55:10:35, v/v/v), and an aqueous solution (20 mmol/l ammonium acetate, pH 4.28). | The retention time of doxazosin and the internal standard was 2.7 and 1.8 min | Quadrupole MS detection | study of relative bioavailability in human plasma | (Ning Ma *et.al.,*2007) |
| LC-MS/MS | Using an isocratic elution on a C18 column | 0.02% formic acid in acetonitrile and 5 mM ammonium formate with 0.02% formic acid (55:45, v: v) | flow rate of 1.1 mL/min | Positive TurboIonSpray mass spectrometry | Detection of doxazosin and verapamil concentration simultaneously in serum of human | (Lukas Chytil *et.al.,*2010) |
| liquid chromatography–tandem mass spectrometry | Chiral separation on an ovomucoid column was achieved in nine minutes. | a methanol/5 mM ammonium acetate/formic acid isocratic mobile phase (20/80/0.016, v/v/v) | flow rate of 0.60 mL/min. | chiral chromatography with MS/MS detection. | To estimate the concentration of doxazosin enantiomers in plasma of humans. | (Ke Liu *et.al.,* 2010) |
| High performance liquid chromatography mass spectrometric method | A 150 x 4.6 mm, 5 µm, 100 A° unisol C18 column | Acetonitrile and a 0.05 percent ammonia solution in HPLC-grade water with isocratic elution make up the mobile phase. | a flow rate of 1.000 mL/minute. elution time for doxazosin and internal standard were 2 min. | liquid chromatography–tandem mass spectrometry with positive electro spray ionization | Detection of doxazosin in plasma of humans (K2EDTA) | (Kapri, A *et.al.,*2019) |

| | | | | | | |
|---|---|---|---|---|---|---|
| high-performance liquid chromatographic quantification | In 8.0 minutes, a reverse-phase Capcell-Pak C18 column (150 × 4.6 mm i.d., 5 µm) produced a chromatographic separation. | Methanol and water with 10 mM perchloric acid and 1.8 mM sodium heptane sulfonic acid (50:50, v/v) made up the mobile phase. ) | flow rate of 1.5 mL/min | fluorescence detection | determination of doxazosin in plasma of humans | (Young Jae Kim et.al., 2006) |
| reversed phase liquid chromatography-tandem mass spectrometry | An XTerra MS $C_{18}$ column | a gradient elution mobile phase made of acetonitrile and 2 mM ammonium acetate | flow rate of 400 µL/min | LC-MS-MS with positive electrospray ionization | doxazosin determination in canine plasma | (Marijana Erceg *et.al.,* 2010) |

## CONCLUSION

Doxazosin, a widely used antihypertensive and benign prostatic hyperplasia drug, has been the subject of extensive analytical and bioanalytical investigation due to its clinical importance and complex pharmacokinetic profile. This review has highlighted a variety of techniques employed for its qualitative and quantitative determination, including spectrophotometry, high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and other emerging analytical platforms. Each method offers unique advantages in terms of sensitivity, specificity, and applicability to different matrices, from bulk drug formulations to biological fluids such as plasma and urine. Bioanalytical methods, particularly LC-MS/MS, have proven essential for pharmacokinetic, bioequivalence, and therapeutic monitoring studies. Recent advances continue to improve method efficiency, environmental sustainability, and regulatory compliance. Future trends may involve the integration of green chemistry principles, automation, and miniaturization to further enhance the analytical landscape for doxazosin. Ultimately, the continual refinement of these methods plays a crucial role in ensuring the drug's safety, efficacy, and quality in clinical and pharmaceutical settings.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

Not applicable

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## AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

## DATA AVAILABILITY

Data will be available on request

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